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Progress Report
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Systematic Description and Key to
Isolants from Chile-Atacama Desert
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Preface

The following report identifies isolants from the soils of the Chile-Atacama Desert. The report includes a "List of the Species Designations" and a "Dichotomous Key" for all the isolants. Descriptive Charts and Tables of Reactions for the viable bacterial isolants are included and followed by photomicrographs of the nonviable cultures. Because of the presence of organisms generally identified as "soil diphtheroids", some of the aspects of their classification are presented.

Yeast and mold isolants were disregarded.

Species identification of the actinomycete isolants were presented in the reports, Systematic Description and Key to Streptomyces Isolants from Chile, Mexico and Arizona Desert Soils, August 1968, and Systematic Description and Key to Streptomyces Isolants from Chile, Arizona and Antarctic Desert Soils, February 1969, by Professor W.B. Bollen and Sumie Nishikawa. The Nocardia species, which are included with the classification of actinomycetes, are presented here with the eubacteria because of their inclusion in the "soil diphtheroids".

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INTRODUCTION

The use of the term "soil diphtheroids" was first used by Bisset and Moore (1949) to describe a group of unicellular microorganisms. They emphasized the unicellularity or multicellularity of the bacterial species and suggested the genus name of Jensenia to represent the unicellular organisms with occasional branching or formation of an early unstable mycelium. They considered the genera Corynebacterium, Mycobacterium, and Nocardia to be multicellular. Their "confusing" definition of Jensenia was not clarified with any references to earlier workers nor any comparisons with isolants from other laboratories. More recently various workers have established Bisset and Moore's Jensenia as the same as a Nocardia sp.¹

Our frequent references to the "soil diphtheroids" has been a designation of convenience due to the existing controversial and inadequately delineated classification schemes for these pleomorphic soil organisms. The designation "soil diphtheroids" has been used to describe the aerobic, Gram-positive, nonsporeforming rods, that are irregularly shaped cells varying from long, club- or wedge-shaped rods, which may exhibit branching (sometimes approaching small mycelia), to short almost coccoid elements. The rods are often arranged in angular form-

¹Clark (1952) found the type species, Jensenia canicruria (Bisset and Moore, 1950), identical with a culture described by Topping (1934) as a Nocardia (Proactinomyces). Adams and McClung (1960) using electron micrographs and cell-wall preparations with the light microscope observed the morphological similarity of Jensenia canicruria to Nocardia rubra (Krasil'nikov) Waksman and Henrici. Gordon and Mihm (1957, 1959) and Gordon (1966) assigned Nocardia rubra (Krasil'nikov) to Mycobacterium rhodochrous. Gordon and Mihm (1961) examined the physiological characteristics of Jensenia canicruria and found it synonymous with Mycobacterium rhodochrous. Jones and Bradley (1964) found that on the basis of susceptibility to actinophage Jensenia was synonymous with Nocardia.

ations and parallel bundles of cells. Previous workers have classified these soil organisms in various genera such as Corynebacterium, Mycobacterium, Nocardia (Proactinomyces), Arthrobacter, Brevibacterium, Cellulomonas, Microbacterium, and Mycococcus. To illustrate the ambiguity in classifying these "soil diphtheroids", strains belonging to the Mycobacterium rhodochrous species have been placed in eleven different genera by different investigators (Gordon, 1966).

Through the ~~past~~ 50 years these genera have been variously placed in different classification schemes. An example of the controversy in classification is shown by the following: the closely related corynebacteria and mycobacteria were originally included in the actinomycetes by Jensen (1931); Stanier and vanNiel (1941) thought they should be excluded; then in 1943, Waksman and Henrici included Mycobacterium with the actinomycetes and not the Corynebacterium. The latter situation is their present position in Bergey's Manual of Determinative Bacteriology (Breed, et. al., 1957). Recent analyses by Cummins (1962) of the cell-wall compositions and antigenic cross-agglutination reactions of Corynebacterium, Mycobacterium, and Nocardia have shown the same characteristic cell-wall composition in each genera. This collaborates many of the earlier workers in their groupings of the genera together.

Various phylogenic schemes have been proposed showing the relationships of the "soil diphtheroids" to the actinomycetes and eubacteria. Because of the transitional relationships from the Streptomyces through the Nocardia to the corynebacteria and mycobacteria and because of the occurrence of these organisms in the Chile-Atacama Desert soils, some of the characteristics and relationships of the Nocardia are discussed here (the other types of soil diphtheroids will be discussed in a later report).

Nocardia

The first recorded observation of actinomycetes was by Cohn (1875) in which he described and illustrated fragmentation and branching. The varying designations and opinions of the earlier workers on the classification of these organisms makes reviewing of the earlier literature difficult. Previous classification schemes are reviewed by Waksman (1959, 1961, 1967). The earlier workers emphasized structural characteristics in classifying Nocardia; consequently, separation of various species is based primarily on these morphological considerations. This type of separation causes difficulty because little work has been done on the structural differences commonly observed in otherwise similar strains of Nocardia.

Nocardia are transitional microorganisms between the streptomycetes on one hand and the mycobacteria and corynebacteria on the other. The separation of the Nocardia from its neighboring genera is difficult because the distinctive characters are shared by each genus. The characterization of a species becomes more quantitative rather than qualitative in nature and those questionable or "borderline" strains become an arbitrary placement.

Separation of Nocardia from Streptomycetes. The morphological separation of Nocardia from Streptomyces is based upon the fragmenting mycelium of Nocardia and nonfragmenting mycelium of Streptomyces. Streptomyces produce spore-like structures on aerial hyphae whereas in Nocardia the aerial hyphae do not differ from the vegetative mycelium. In some cases these characters overlap: there are Streptomyces which lose their ability to form aerial hyphae and Nocardia which form various degrees of an aerial hyphae. Nocardia reproduce by fragmentation of the mycelium and not by conidia (Adams and McClung, 1962a).

Further separation among the two genera can be exhibited in their biochemical properties. Most Streptomyces hydrolyze starch and casein, liquefy gelatin and produce diffusible pigments whereas most Nocardia do not (Jones and Bradley, 1964). Jones and Bradley (1964) observed that in milk the oxidation-reduction potential of Streptomyces was lowered (E_h 0 to -200millivolts) whereas the nocardial and mycobacterial potential was high (E_h 50 to 180mV) like the uninoculated medium (E_h 150mV). They also tested the susceptibility of actinomycetes to antibiotics and found of those drugs tested only one, endomycin, significantly differentiated these organisms: nocardia were inhibited by 5-25 μ g/ml. and mycobacteria and streptomycetes were resistant to 250 μ g/ml.

Another criterion for distinguishing between Streptomyces and Nocardia is the chemical composition of their cell walls. Nocardial cell walls contain arabinose, whereas those of Streptomyces do not contain a characteristic sugar (Cummins and Harris, 1958; Romano and Sohler, 1956). In Nocardia DL-diaminopimelic acid is found whereas in Streptomyces LL-diaminopimelic acid is found (Hoare and Work, 1957). Tewfik and Bradley, (1967) studying the deoxyribonucleic acids from Streptomyces and Nocardia, found the DNA of Streptomyces to contain between 69 and 73% G + C. The DNA of the Nocardia formed two groups, 62-64% G + C and 68-69% G + C.

Separation of Nocardia from Mycobacteria and Corynebacteria. The separation of Nocardia from the mycobacteria and corynebacteria is difficult because the distinguishing characters are shared by each genus and there are more similarities than dissimilarities. The morphological separation is based upon the formation of an initial mycelium in the Nocardia and its absence in Mycobacterium and Corynebacterium. Overlapping occurs and initial mycelium in Nocardia may be small and transient whereas some mycobacteria and corynebacteria produce a rudimenta-

ry mycelium. Presence of small granules resembling beginnings of aerial hyphae have been observed in mycobacteria and corynebacteria (Jensen, 1934; Lacey, 1955). All three genera exhibit multiple fragmentation (Bisset, 1949; Adams and McClung, 1962a). Also, "spore-like bodies" that germinate in fresh media with one or more slender sprouts are found in each genera.

The Mycobacterium are generally considered to be acid-fast, the Nocardia partially acid-fast, and the Corynebacterium nonacid-fast. There are reports of acid-fastness in the Corynebacterium (Jensen, 1934). Acid-fastness is variable and often not demonstrable in Mycobacterium. Acid-fastness of a culture is not a reliable character because it depends upon the length of time since isolation as well as composition of the medium, temperature of incubation and age of the culture (McClung and Uesaka, 1960). Metachromatic granules, commonly used to characterize Corynebacterium, have been demonstrated in Nocardia (McClung, 1950; Adams and McClung, 1962b).

Separation of these genera on biochemical bases is also undistinctive. One group of nocardia are similar to corynebacteria. They are nonacid-fast and have less refractive cells. They possess a generally more fermentative metabolism, lack ability to utilize paraffin, and frequently possess diastatic and proteolytic enzymes. The other group of nocardia are similar to mycobacteria. They are acid-fast and have refractile cells. They utilize paraffin and other carbon compounds, have an oxidative metabolism, and lack diastatic and proteolytic enzymes. They also render milk partly transparent without digestion of the casein (Jensen, 1953). The pigments of the acid-fast, nonproteolytic, oxidative nocardia are orange or red; whereas, the nonacid-fast, proteolytic, fermentative nocardia are yellow, greenish, or non-pig-

mented (Krasil'nikov, 1938a).

As mentioned before, studies on the chemical composition of the cell walls and serological reactions of Nocardia, Mycobacterium and Corynebacterium support the similarity of these genera (Cummins, 1962; Slack, Winger and Moore, 1961). Separation may be made on basis of different infrared spectra of whole cells (Arai, Kuroda, and Koyama, 1963). The difference in spectra are a reflection of the differences in lipid content of these organisms. The genus Mycobacterium may be defined on the basis of its characteristic branched fatty acids. As-selineau (1962) reports that characteristic lipids are found in mycobacteria with about 80 carbon skeletons (mycolic acids), in nocardia with about 50 carbon skeletons (nocardic acids), and in corynebacteria with about 32 carbon skeletons (corynomycolic acids). Lan  elle, et. al. (1965) found mycolic acids different from nocardic acids. Mycolic acids can be extracted with chloroform whereas nocardic acids cannot. Pyrolysis of mycolic acids liberates tetracosanoic acid or hexacosanoic acid, whereas pyrolysis of nocardic acid liberates palmitic or stearic acids. Lan  elle also indicates nocardia have few waxes (chloroform-soluble lipids) and mycobacteria have many.

Wayne and Gross (1968) studied the deoxyribonucleic acid base composition of mycobacteria and nocardia. They reported that the two genera could not be separated on basis of DNA base composition.

The preceding gives an indication of the difficulties in separating Nocardia from its neighboring genera and illustrates its transitional nature. To further illustrate the variances among the nocardia various workers have divided the group into parts based on different properties: acid-fastness vs. nonacid-fastness, stable mycelium vs. unstable mycelium, proteolytic vs. nonproteolytic, aerial mycelium vs. no aerial

mycelium. Unfortunately the properties used to divide the nocardia into groups do not coincide with each other; e.g., there are acid-fast nocardia with stable mycelium and acid-fast nocardia with unstable mycelium.

General Definition of Nocardia. The general definition of Nocardia distinguishes them morphologically by indicating their early formation of a transient nonseptate mycelium. Upon aging this mycelium breaks into short, irregular cylindrical cells. Upon further aging the short cylindrical cells become small coccoid elements (see nigrosin-stain photomicrographs for isolant number 245a-2 of this report). Swollen cells which form "germ-tubes" upon transfer to fresh medium are produced. The external structure and appearance of Nocardia colonies may be smooth or rough and much folded. The consistency of the colonies may be soft and dough-like or compact and leathery. Also, aerial hyphae that are usually indistinguishable from the substrate mycelium may be formed.

The extent of initial mycelium formation varies within the nocardia. Variation occurs from a strain forming an extensive mycelium, no fragmentation, and contorted and overlapping branches to one forming rudimentary mycelium, early fragmentation, and sparse branching. The rudimentary mycelium may also be described as elongated irregular rods with a few short lateral branches. McClung (1949) suggested the term "mycelium" be defined as a branched hyphae at least 10 microns long. Branches are simple lateral protuberances from a hyphae.

Fragmentation is another phenomenon found in nocardia. The term describes a process of cell division in surface or sub-surface mycelium. It occurs as a result of septation before cell division; the result is the breaking up of mycelium into small rods or coccoid cells

(Adams and McClung, 1962a). McClung (1949) reports three types of fragmentation occurring in nocardia: "Type 1" is distinguished by the formation before division of an acute angle in the hyphae at the apex of the bend. After division, the ends of the two new cells grow out parallel to each other (they rarely cross). In "Type 2" fragmentation division occurs in a straight or slightly curved portion of a hypha, then the newly formed ends bend slightly away from each other and continue to grow. In "Type 3" fragmentation, the division of the parent hypha occurs near or at the juncture of a branch. The end of the newly formed cell bends slightly and continues to grow. At the place of division of the parent hypha a new branch may grow.

Mycelium development in the "extensive-mycelium" group is more stable and resembles Streptomyces-like mycelium. The mycelium development in the "rudimentary-mycelium" group is unstable and resembles corynebacteria and mycobacteria. McClung (1949) found that the longer the period of growth before fragmentation, the greater the amount of mycelium present. Using permanency of the mycelium as the basis, he established three groups in Nocardia:

"Group I" is characterized by very early fragmentation, sparse branching, and limited mycelial development. Many coccoid elements occur in old material. The texture of the colonies is soft, pasty, and sometimes mucoid. The pigments are intracellular and insoluble. "Type 1" fragmentation is characteristic of this group and similar to cell division in corynebacteria.

"Group II" is characterized by a delayed fragmentation, extensive mycelial development, and straight branches which do not overlap. Fragmentation is "Type 3". The colonial texture is soft and pasty. The pigment is intracellular and insoluble.

"Group III" is characterized by the absence of fragmentation and presence of profusely produced overlapping and contorted branches. The colonies are either waxy (can be separated into flakes by a needle) or cartilaginous (cannot be separated by the needle). In general both intracellular and soluble pigments are produced.

Fragmentation "Type 1" is characteristic of "Group I", "Type 3" is characteristic of "Group II", and "Type 2" occurs in both groups.

"Group III" has no fragmentation.

Adams and McClung (1960, 1961, 1962a) have studied the developmental cycle of Nocardia. They found that all cell fragments (bacillary, ovoid, or coccoid) of the inoculum begin growth by formation of germ tubes. Upon continued growth of the germ tubes filamentous cells result. The next phase, branching, is a result of three processes: 1. Simple branch formation from the filamentous germ tubes, 2. Branch formation as a result of multiple germination of the cells (more germ tubes produced from original cell), and 3. Single or multiple germination of fragments of incompletely separated chains. After the branching phase is completed, fragmentation occurs. With increased age of the culture, more septa are deposited and the resulting filament fragments become shorter until the completion of the cycle. Some of the fragments may "round up" and form resistant cells called microcysts.

The presence of nuclei and life-cycles in nocardia is still undecided (McClung, 1962). In addition to metachromatic granules, lipid inclusions have been observed in nocardia (McClung, 1962). Motility is present in nocardia and is thoroughly discussed by Jensen (1953).

Nocardia of Chile-Atacama Desert. The organisms classified as Nocardia in this report are of the soft bacterial type. The cells are not acid-fast nor very refractile. Their metabolism is fermentative,

they do not utilize paraffin, but they hydrolyze starch and gelatin, Although, the biochemical reactions are the same for all six, they differ in cultural appearances and mycelium formation.

Cultures 245a-1 and 245a-2 differ from each other in color; 245a-2 is a very bright yellow, whereas 245a-1 is a very light yellow. This phenomenon is probably due to dissociation. These two cultures differ from the other four Nocardia spp. in that they form a more extensive mycelium. The mycelium is demonstrated in the photomicrographs for 245a-2 and in the photomicrographs of colony morphology for 245a-1 and 245a-2. The colony margins of 274b-1, 274b-2, 275a-1, and 275b are entire.

Isolants 274b-1 and 274b-2 are different only in the topography of their colonies. 274b-2 is rough and wrinkled whereas 274b-1 is smooth. This also was probably a result of dissociation. Isolant 275a-1 appears the same as 274b-2.

Isolant 275b is lighter in color than the other isolants except 245a-1. It is interesting to note that 275b and 245a-1 have a very light yellow pigment but differ in the extent of mycelium development. Isolants 245a-2, 274b-1, 274b-2, and 275a-1 all have the same bright yellow pigment production. The topography of the colonies of 245a-2 and 274b-1 were both smooth; they differ only in extent of mycelium development.

Studies of the fragmentation and developmental cycles of these species have not been made, but developing cells (photomicrograph for 274b-2) and shortening of cells upon increasing age (photomicrographs for 275b, 275a-1, and 245a-2) can be seen.

Mycococcus, Mycobacterium, Arthrobacter and Bacillus

A discussion of the placement of isolants 274a with the Mycococcus (Krasil'nikov, 1938b), 248b, 248Aa-1 and 248Aa-2 with the Mycobacterium (Jensen, 1934; Krasil'nikov, 1938c, 1941) and 247Bb with the Arthrobacter will be included in a later report.

The Bacillus spp. are again classified using the dichotomous key of Smith, Gordon and Clark (1952) and Bergey's Manual of Determinative Bacteriology (Breed, 1957).

LIST OF ISOLANTS AND SPECIES DESIGNATIONS

<u>Code</u> <u>Number</u>	<u>Species</u> <u>Designations</u>	<u>Page</u>
<u>Van Delden's Medium (#6):</u>		
245a-1	<u>Nocardia</u> sp.	25
245a-2	<u>Nocardia</u> sp.	23
248b	<u>Mycobacterium</u> sp.	35
275a-1	<u>Nocardia</u> sp.	27
275a-2	<u>Bacillus firmus</u> .	59
276a	Actinomycete (#36. <u>Streptomyces albus</u>).	
277a-1	<u>Bacillus badius</u> .	55
277a-2	<u>Bacillus coagulans</u> .	53
<u>Van Delden's Medium from Boyd's - D Medium (#6-20d):</u>		
248a	<u>Bacillus pumilus</u> .	51
274a	<u>Mycococcus capsulatus</u> .	43
274b-1	<u>Nocardia</u> sp.	31
274b-2	<u>Nocardia</u> sp.	29
275a	<u>Bacillus firmus</u> .	57
275b	<u>Nocardia</u> sp.	33
<u>Nitrate agar (#24):</u>		
*245Aa	(Soil Diphtheroid).	61
245Bb	Actinomycete (#23. <u>Streptomyces griseoviridis</u>).	
245Bc	Actinomycete (#24. No Growth).	
245Bd	Mold.	
*245Be	(Soil Diphtheroid).	61
246Aa	Mold.	

*Fail to grow upon original transfer.

246Bb	Actinomycete (#28. <u>Streptomyces caelestis</u>).	
246Bd	Actinomycete (#29. <u>Streptomyces caelestis</u>).	
246Ae	Actinomycete (#26. <u>Streptomyces lavendulae</u>).	
246Af	Actinomycete (#27. <u>Streptomyces collinus</u>).	
247Bb	<u>Arthrobacter</u> sp.	41
247Bc	Actinomycete (#31. <u>Streptomyces tendae</u>).	
247Ad	Actinomycete (#30. No Growth).	
248Aa	Actinomycete (#32. <u>Streptomyces longisporuber</u>).	
*248Ab	(Soil Diphtheroid).	61
248Ac	Mold.	
249Aa	Actinomycete (#33. <u>Streptomyces antibioticus</u>).	
249Ab	Actinomycete (#34. No Growth).	
<u>Stephenson's A Medium (#25):</u>		
245a	Actinomycete (#22. <u>Streptomyces netropsis</u>).	
246Aa	Actinomycete (#25. No Growth).	
*246b	(Soil Diphtheroid).	62
248Aa-1	<u>Mycobacterium</u> sp.	37
248Aa-2	<u>Mycobacterium</u> sp.	39
248Ab	Mold.	
260a	<u>Bacillus cereus</u>	47
<u>Stephenson's B Medium (#26):</u>		
246a	<u>Bacillus cereus</u>	45
259a	Yeast.	62
*260a	(Soil Diphtheroid).	62
260b	<u>Bacillus</u> sp.	49

*Fail to grow upon original transfer.

DICHOTOMOUS KEYYEAST AND MOLDS:

- 245Bd (#24) Mold.
- 246Aa (#24) Mold.
- 248Ac (#24) Mold.
- 248Ab (#25) Mold.
- 259 a (#26) Yeast.

STREPTOMYCETES:*

- 276 a (# 6) (#26. Streptomyces albus).
- 245Bb (#24) (#23. Streptomyces griseoviridis).
- 245Bc (#24) (#24. No Growth).
- 246Bb (#24) (#28. Streptomyces caelestis).**
- 246Bd (#24) (#29. Streptomyces caelestis).
- 246Ae (#24) (#26. Streptomyces lavendulae).
- 246Af (#24) (#27. Streptomyces collinus).
- 247Bc (#24) (#31. Streptomyces tendae).
- 247Ad (#24) (#30. No Growth).
- 248Aa (#24) (#32. Streptomyces longisporuber).**
- 249Aa (#24) (#33. Streptomyces antibioticus).
- 249Ab (#24) (#34. No Growth).
- 245 a (#25) (#22. Streptomyces netropsis).
- 246 a (#25) (#25. No Growth).

*Identified in report by W.B. Bollen and Sumie Nishikawa, August 1968.

**Identified in report by W.B. Bollen and Sumie Nishikawa, February 1969.

BACTERIA:*

Non-viable.

245Aa (#24)
 245Be (#24)
 248Ab (#24)
 246b (#25)
 260a (#26)

Viable.

Gram-positive rods.

I. "SOIL DIPHTHEROIDS".

Do not produce endospores.

- A. Branched nonseptate mycelium in early stages of growth.
 Mycelium breaks into short cylindrical cells which later become coccoid.
 Fragmentation present.
 Slight to moderate production of catalase.
 Slight acid production in litmus milk.
 Do not utilize asparagine nor ammonium as sole nitrogen sources.
 Growth better on trypticase soy agar and nutrient agar without 1% glucose added.
 Fermentative (anaerogenic) metabolism.

NOCARDIA.

1. Margin fimbriate.
 Branching mycelium present.
 Aerobic.
 Nutrient gelatin not liquefied.

- a. Honey Gold (2ic) to Gold (1½pc) pigmentation.
 Nonmotile.

245a-2 (#6)

- b. Bamboo (2gc) to Mustard (2le) pigmentation.
 Motile.

245a-1 (#6)

2. Margin entire.
 Fragmentation present.

*The genera Nocardia, Mycobacterium, and Mycococcus (classified in Bergey's Manual with Actinomycetales) are included.

Aerobic to slightly facultative.
Nutrient gelatin slowly liquefied.

- a. Motile.
Honey Gold (2ic) to Gold ($1\frac{1}{2}$ pc) pigmentation.

(1). Topography rough.

(a). Growth on urea hydrolysis agar.

275a-1 (#6)

(b). No growth on urea hydrolysis agar.

274b-2 (#6-20d)

(2). Topography smooth.
No growth on urea hydrolysis agar.

274b-1 (#6-20d)

- b. Nonmotile.
Bamboo (2gc) to Lt. Yellow ($1\frac{1}{2}$ ea) pigmentation.

275b (#6-20d)

- B. Mycelium or branching not present.
Size and shape of the rods vary.
Cells are nonmotile.
Involution forms occur.
Utilize asparagine and ammonium as sole nitrogen sources.
Growth is better on trypticase soy agar and nutrient agar with 1% glucose added.

1. Angular arrangements and parallel bundles of cells are common.
The rods may be slightly bent, may occur in wedge shapes and may exhibit clubs.
The width of cells is 0.4 to 0.6 μ and the average length diminishes with age (young cells 1.5-2.0 μ , old cells 0.8-1.0 μ).
Strong catalase production.
Reaction in litmus milk is alkaline.
Urea is hydrolyzed.
2% NaCl tolerated.
Growth at 40°C. but not 45°C.

- a. Red to orange-red non-water-soluble pigment produced.
No arthrospores present.
Gelatin not hydrolyzed nor liquefied.
Casein not hydrolyzed.
Citrate not utilized.
10% NaCl tolerated.
Tellurite reduced.
Nitrate not utilized as sole nitrogen source.

Nonoxidative-nonfermentative metabolism.

MYCOBACTERIUM.

- (1). Diameter of colonies 2-4mm., shape irregular, elevation convex, and texture very moist and mucoid.

248b (#6)

248Aa-1 (#25)

- (2). Diameter of colonies 0.5mm., shape puntiform, elevation pulvinate, and texture more compact and drier than above.

248Aa-2 (#25)

- b. No pigment produced, culture white.
Arthrospores present.
Gelatin hydrolyzed and liquefied.
Casein hydrolyzed.
Citrate utilized as sole carbon source.
7% NaCl not tolerated.
Tellurite not reduced.
Nitrate utilized as sole nitrogen source.
Growth at 35°C., but not 40°C.
Oxidative metabolism.

ARTHROBACTER.

247Bb (#24)

2. Cells not arranged angularly nor in parallel bundles.
Cells ovate, coccoid, and rod (length not exceeding twice the width) shaped. Many cells in shape resemble pears and bowling pins.
Greatly enlarged cells and minute cells are present in the same culture.
The width of the cells varies from 0.74-1.25 μ (may be as large as 2.2 μ in involution forms) and averages 0.8 μ .
The average length is 1.2 μ .
Slight catalase production.
No reaction in litmus milk.
Urea not hydrolyzed (no growth on medium).
2% NaCl not tolerated.
No reaction in oxidation-fermentation medium.

MYCOCOCCUS.

274a (#6-20d)

- II. Produce endospores.
Survive pasteurization (85°C., 10 minutes).

Genus BACILLUS.

Remaining key is taken from Breed (1957) and Smith, Gordon, and Clark (1952).

- I. Sporangia not definitely swollen.
Spores ellipsoidal to cylindrical, central to terminal.
Spore walls thin and not easily stained.
- A. Diameter of vegetative rods is 0.9 micron or more.
1. Acid from mannitol with ammonium salts as source of nitrogen.
Acetylmethylcarbinol not produced.
 1. Bacillus megaterium.
 2. No acid from mannitol with ammonium salts as source of nitrogen.
Acetylmethylcarbinol produced.
 2. Bacillus cereus.
 - (a. Urea hydrolyzed.
 - 246a (#26) b. Urea not hydrolyzed.
 - 260a (#25)
 3. No acid from mannitol with ammonium salts as source of nitrogen.
Acetylmethylcarbinol not produced.)*

Bacillus sp.

 - 260b (#26)
- B. Diameter of vegetative rods is less than 0.9 micron.
1. Growth on glucose agar as good or better than on agar.
Good growth on soybean agar.
 - a. Growth on 7 percent NaCl agar.
 - b. Starch hydrolyzed.
Nitrites produced from nitrates.

*Not a part of the dichotomous keys of Breed (1957) or Smith, Gordon and Clark (1952).

5. Bacillus licheniformis.

6. Bacillus subtilis.

bb. Starch not hydrolyzed.
Nitrites not produced from nitrates.

7. Bacillus pumilus.

248a (#6-20d)

aa. No growth on 7 percent NaCl agar.

b. Glucose utilized.
Weak, if any, hydrolysis of gelatin.

8. Bacillus coagulans.

277a-2 (#6)

bb. Glucose not utilized.
Strong hydrolysis of gelatin.

9. Bacillus badius.

277a-1 (#6)

2. Growth on glucose agar definitely not so good as on agar.
Scant, if any, growth on soybean agar.

a. Casein hydrolyzed. Urease not produced.

10. Bacillus firmus.

275a (#6-20d)

275a-2 (#6)

aa. Casein not hydrolyzed. Urease produced.

11. Bacillus lentus.

TABLE 1: PHYSIOLOGICAL REACTIONS

CODE NUMBERS:	HYDROLYSES:						Indol Production	Acetylmethylcarbinol Production	REDUCTIONS:	
	Gelatin	Starch	Casein	Urea	Fat	Cellulose			Nitrate	Tellurite

Soil diphtheroids:

245a-2	+	+	-	-	-	-	-	-	+	-
245a-1	+	+	-	-	-	-	-	-	+	-
275a-1	+	+	-	-	-	-	-	-	+	-
274b-2	+	+	-	-NG	-	-	-	-	+	-
274b-1	+	+	-	-NG	-	-	-	-	+	-
275b	+	+	-	-	-	-	-	-	+	-
247Bb	+	+	+	+	-	-	-	-	+	-
248b	-	s	-	+	-	-	-	-	+	+
248Aa-1	-	s	-	+	-	-	-	-	+	+
248Aa-2	-	s	-	+	-	-	-	-	+	+
274a	-	s	-	-NG	-	-	-	-	s	-

Bacillus spp.:

246a	+	+	+	+		-	-	+	-	
260a	+	+	+	+			-	+	+	
260b	+	+	-	-		-	-	-	+	
248a	+	-	+	-			-	+	-	
277a-2	-	+	-	-		-	-	-	+	
277a-1	+	-	-	-NG		-	-	-	+	
275a	+	+	-	-NG		-	-	-	+	
275a-2	s	+	-	-NG			-	-	+	

s = slight.

NG = no growth.

TABLE 2: PHYSIOLOGICAL REACTIONS

CODE NUMBERS:	UTILIZATIONS					LITMUS MILK*		Anaerobic Nitrate Broth Gas	Soy Bean Infusion Agar**	Fat Agar**	Glucose Nutrient Agar vs. Nutrient Agar	Oxygen Relationship***	Catalase Production
	Asparagine	Ammonium	Nitrate	Citrate	Paraffin	Peptonization	Reduction						
						Reaction	Curd						

Soil diphtheroids:

245a-2	-	-	-	-	-	-	s.ac.	-	-	M	A	NA	A	+
245a-1	-	-	-	-	-	-	s.ac.	-	-	M	A	NA	A	+
275a-1	-	-	-	-	-	-	s.ac.	-	-	M	A	NA	AF	+
274b-2	-	-	-	-	-	-	s.ac.	-	-	M	A	NA	AF	+
274b-1	-	-	-	-	-	-	s.ac.	-	-	M	A	NA	AF	+
275b	-	-	-	-	-	-	s.ac.	-	-	M	A	NA	MA	+
247Bb	+	+	+	+	-	c	+ alk	-	-	A	A	GNA	A	+
248b	+	+	-	-	+	-	s alk	-	-	A	A	GNA	A	+
248Aa-1	+	+	-	-	+	-	s alk	-	-	A	A	GNA	A	+
248Aa-2	+	+	-	-	+	-	- alk	-	-	A	A	GNA	A	+
274a	s	s	s	s	s	-	-	-	-	A	A	GNA	M	+

Bacillus spp.:

246a	+	s	+			+	+ alk	-	-	A		GNA	A	+
260a	+	+	+			+	+ alk	-	-	A		GNA	A	+
260b	+	-	+			-	-	-	-	A		NA	A	+
248a	s	+	+			+	s alk	-	-	A		NA	A	+
277a-2	-	-	-			-	-	-	-	M		NA	M	+
277a-1	-	-	-			-	-	-	+	-		NA	A	+
275a	-	-	-			-	-	-	-	-		NA	F	+
275a-2	-	-	-			-	-	-	-	-		NA	AF	+

*Litmus Milk: ac = acid; alk = alkaline; c= clearing, but not peptonization.

**Soybean infusion agar & Spirit blue fat agar: A = abundant; M = moderate; s = slight.

***Oxygen Relationship: A = aerobic; AN = anaerobic; F = facultative; M = microaerophilic.

TABLE 3: TEMPERATURE AND SALT TOLERANCES

CODE NUMBERS:	SALT TOLERANCES:			TEMPERATURES:							
	2%	7%	10%	5°C	10°C	15°C	20°C	40°C	45°C	50°C	55°C
Soil diphtheroids:											
245a-2	+	+	-	-	+	+	+	+	+	-	-
245a-1	+	+	-	-	+	+	+	+	+	-	-
275a-1	+	-	-	-	+	+	+	+	+	-	-
274b-2	+	-	-	-	+	+	+	+	+	-	-
274b-1	+	-	-	-	+	+	+	+	+	-	-
275b	+	-	-	-	+	+	+	+	+	-	-
247Bb	+	-	-	+	+	+	+	-	-	-	-
248b	+	+	+	-	+	+	+	+	-	-	-
248Aa-1	+	+	+	-	+	+	+	+	-	-	-
248Aa-1	+	+	+	-	+	+	+	+	-	-	-
274a	-	-	-	-	-	s	+	+	-	-	-
<u>Bacillus</u> spp.:											
246a	+	+	-	-	+	+	+	+	+	-	-
260a	+	+	-	-	+	+	+	+	+	-	-
260b	-	-	-	-	+	+	+	+	+	-	-
248a	+	+	+	-	+	+	+	+	+	+	-
277a-2	+	+	-	-	+	+	+	+	+	-	-
277a-1	+	-	-	-	+	+	+	+	+	-	-
275a	+	+	-	-	+	+	+	+	+	-	-
275a-2	+	+	-	-	+	+	+	+	+	-	-

Descriptive Chart

245a-2 (#6)
(code number)

Trypticase Soy Agar
(medium)

Chile Atacama Desert
(source)

Nocardia sp.
(name of organism)

28°C.
(temperature)

W.B. Bollen & K.M. Kemper
(studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round & pointed
filaments, cocci, spirals,
branching mycelium

Size: average —
range —

Irregular forms:

GRAM REACTION: Positive.

18 hrs:

24 hrs:

48 hrs:

Negative.
PASTEURIZATION SURVIVAL (85°C, 10 minute
Sporangia: none, rods, spindles, elliptical, clavate,
Endospores: swollen, not swollen.

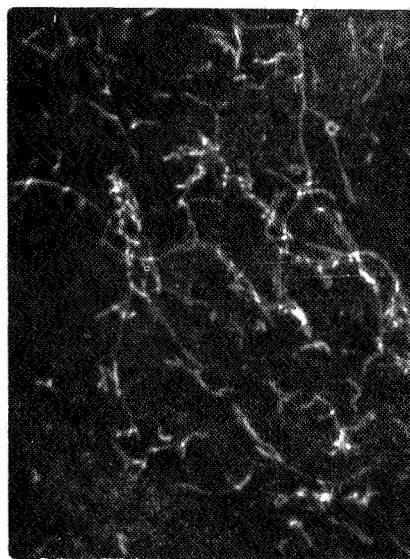
Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —
range —

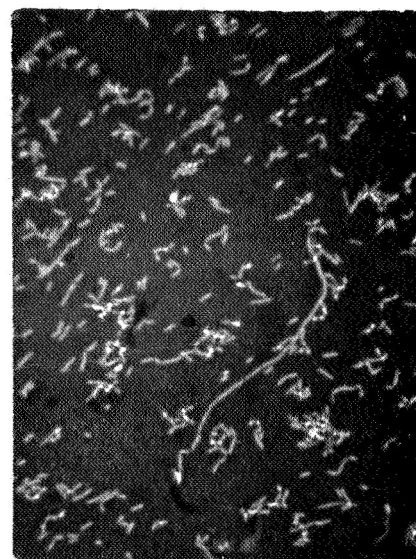
MOTILITY: age 24 hr. Negative.

Flagella:



1000X

48 hr.

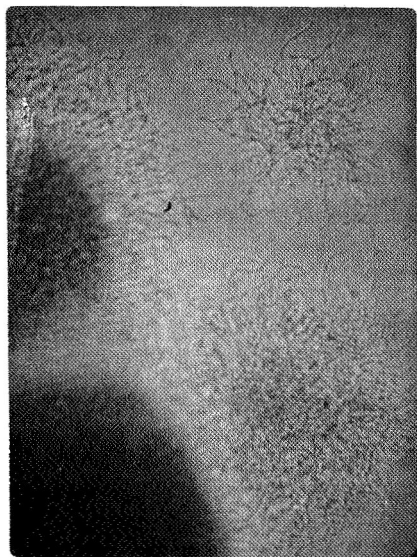


1000X

24 hr.

NIGROSIN STAINS:

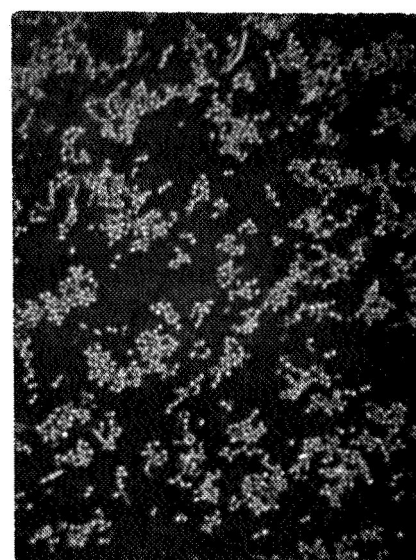
6 da.



400X



1000X



1000X

COLONY MORPHOLOGY TSA:
24 hr.

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 10 da.
 Amount of growth: abundant, moderate, scant.
 Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.
 Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.

AGAR COLONIES: age 10 da.

Macroscopic

Size: 2 mm.

Shape: filamentous, irregular, oval, puntiform, round.

Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.

Topography: contoured, rough, smooth, striated, wrinkled.

Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate, fimbriate.

Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated, aborescent.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.

Appearance by transmitted light: opaque, translucent, transparent.

Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar Brite Yellow 1 1/2 la

~~ECOLIUM~~ Color varies with age:

- 4 da.: 21c Honey Gold.

- 30da.: 1 1/2 pc Gold.

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.

Surface growth: none, flocculent, membranous, pellicle, ring.

Subsurface growth: none, granular, turbid.

Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____.

GELATIN STAB: age 86 da.

Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.

Rate: fast, moderate, slow.

Line of puncture: villous.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: Slight Growth.

Fat agar: Growth.

Glucose-nitrate agar: No Growth. 20 da.

Growth scant on nutrient agar; no growth on - glucose-nutrient agar.

Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.

CATALASE: positive, negative.

TEMPERATURE RELATIONSHIPS: age 17 da.

Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +, 45°C. +, 55°C. -, 50°C. +, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative.

Sucrose: positive, negative.

Xylose: positive, negative.

Citrate: positive, negative.

NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative. 6 da. 15 da.

REDUCTIONS:

Nitrate: NO₃⁻ +, NH₄⁺ _____, gas _____, negative. 5 da.

Methylene blue: positive, negative.

Selenite: positive, negative, slight. 16 da.

Tellurite: positive, negative. 11 da.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid, alkaline, neutral, gas.

Sucrose: acid, alkaline, neutral, gas.

Lactose: acid, alkaline, neutral, gas.

Xylose: acid, alkaline, neutral, gas.

Mannitol: acid, alkaline, neutral, gas.

Fermentative. Anaerogenic.

HYDROLYSIS:

Gelatin: positive, negative. 6 da.

Casein: positive, negative. 24 da.

Fat: positive, negative. 14 da.

Starch: positive, negative. 13 da.

Cellulose: positive, negative. 111 da.

Urea: positive, negative. 14 da.

TOLERANCES:

Salt: 2% positive, negative. 2 da.

7% positive, negative. 7 da.

10% positive, negative. 14 da.

pH: acid, alkaline.

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral, slight. 76 da.

Curd: acid, alkaline, absent, gas. 76 da.

Peptonization: positive, negative. 76 da.

Reduction: positive, negative. 76 da.

OTHER REACTIONS:

H₂S from _____: positive, negative.

NH₄⁺ from peptone: positive, negative. 12 da.

Acetylmethylcarbinol: positive, negative. 12 da.

Indol: positive, negative.

Methyl red: positive, negative.

Descriptive Chart

245a-1 (#6)
(code number)

Trypticase Soy Agar
(medium)

Chile Atacama Desert
(source)

Nocardia sp.*
(name of organism)

28°C.
(temperature)

W.B. Bollen & K.M. Kemper
(studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round & pointed.
filaments, cocci, spirals,
branching mycelia.

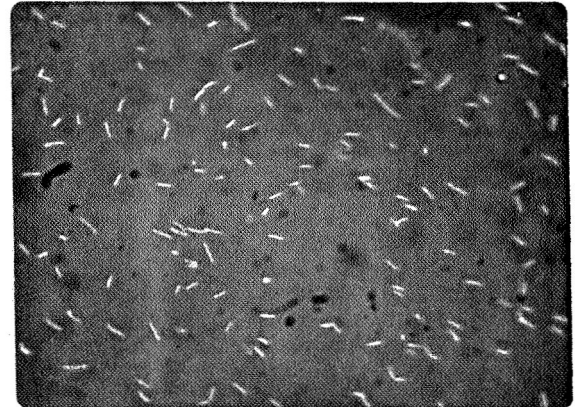
Size: average —
range —

Irregular forms:

GRAM REACTION: Positive.

18 hrs:
24 hrs:
48 hrs:

MIGROSIN STAIN:
24 hr.



1000X

PASTEURIZATION SURVIVAL (85°C, 10 minutes) Negative.

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —
range —

MOTILITY: age 24 hr. Positive.

Flagella:

COLONY MORPHOLOGY TSA 24 HR:

OTHER STAINS:

Acid fast:

Capsule:

Glycogen:

Crystalline dextrans:

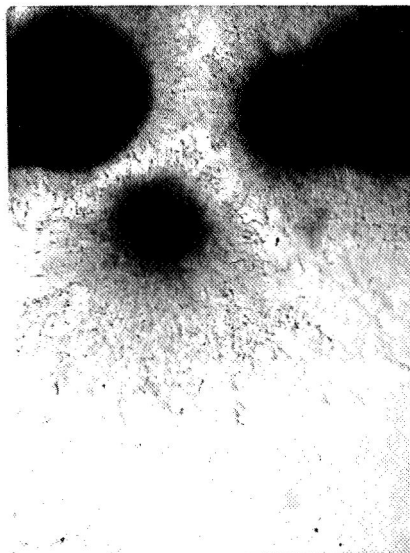
Fat globules:

Metachromatic granules:

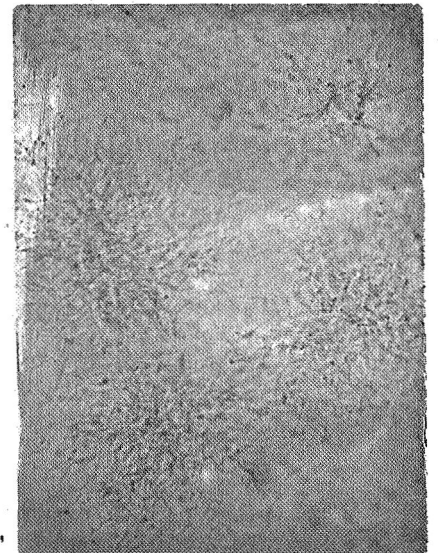
***In slide preparations of various aged cells for microscopic examination, no branching, unseptate mycelia is observed in a young culture; although, colony observations (100X and 400X) made on young cultures show branching mycelia characteristic of Nocardia species.**

When the surface growth of the bacterial colony

is removed, microscopic examination shows substrate mycelia remaining in the agar.



100X



400X

(46)

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 10 da.Amount of growth: *abundant, moderate, scant.*Form: *aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.*Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*AGAR COLONIES: age 10 da.

Macroscopic

Size: 2 mm.Shape: *filamentous, irregular, oval, puntiform, round.*Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*Topography: *contoured, rough, smooth, striated, wrinkled.*Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate. fimbriate.*Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*Appearance by transmitted light: *opaque, translucent, transparent.*Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar lt Yellow 1 1/2 ea~~monotonic~~ Color varies with age:

- 4 da.: 2gc Bamboo.

- 30 da.: 2le Mustard.

NUTRIENT BROTH: age _____

Amount of growth: *abundant, moderate, scant.*Surface growth: *none, flocculent, membranous, pellicle, ring.*Subsurface growth: *none, granular, turbid.*Sediment: *none, compact, flaky, flocculent, granular, viscid.*

Odor: resembling _____

GELATIN STAB: age 86 da.Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*Rate: *fast, moderate, slow.***Line of puncture: villous.**

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar Slight growth.Fat agar: Growth.Glucose-nitrate agar: No growth. 20 da.Slight growth on nutrient agar; no growth on glucose nutrient agar.Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: *aerobic, anaerobic, facultative, micro-aerophilic.*CATALASE: *positive, negative. positive.*TEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +, 45°C. +, 55°C. -, 5°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: *positive, negative.* _____Sucrose: *positive, negative.* _____Xylose: *positive, negative.* _____Citrate: *positive, negative. slight* 11 da.NH₄⁺ AS SOLE NITROGEN SOURCE: *positive, negative.* 15 da.

REDUCTIONS:

Nitrate: NO₃⁻ +, NH₄⁺ _____, gas _____, negative. 5 da.Methylene blue: *positive, negative.* _____Selenite: *positive, negative.* 16 da.Tellurite: *positive, negative.* 11 da.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: *acid, alkaline, neutral, gas.* _____Sucrose: *acid, alkaline, neutral, gas.* _____Lactose: *acid, alkaline, neutral, gas.* _____Xylose: *acid, alkaline, neutral, gas.* _____Mannitol: *acid, alkaline, neutral, gas.* _____Fermentative. Anaerogenic.

HYDROLYSIS:

Gelatin: *positive, negative.* 6 da.Casein: *positive, negative.* 24 da.Fat: *positive, negative.* 14 da.Starch: *positive, negative.* 3 da.Cellulose: *positive, negative.* 111 da.Urea: *positive, negative.* 14 da.

TOLERANCES:

Salt: 2% *positive, negative.* 2 da.7% *positive, negative.* 14 da.10% *positive, negative.* 14 da.pH: *acid, alkaline.* _____

LITMUS MILK REACTIONS:

Reaction: *acid, alkaline, neutral. slight.* 76 da.Curd: *acid, alkaline, absent, gas.* 76 da.Peptonization: *positive, negative.* 76 da.Reduction: *positive, negative.* 76 da.

OTHER REACTIONS:

H₂S from _____: *positive, negative.* _____NH₄⁺ from peptone: *positive, negative.* _____Acetylmethylcarbinol: *positive, negative.* 28 da.Indol: *positive, negative.* 28 da.Methyl red: *positive, negative.* _____

Descriptive Chart

275a-1 (#6)
(code number)

Trypticase Soy Agar
(medium)

Chile Atacama Desert
(source)

Nocardia sp.*
(name of organism)

28°C.
(temperature)

W.B. Bollen & K.M. Kemper
(studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round & pointed.
filaments, cocci, spirals,
branching _____

24 hr.

Size: average —
range —

Irregular forms:

GRAM REACTION Positive.

18 hrs:

24 hrs:

48 hrs:

NIGROSIN STAINS:



1000X

PASTEURIZATION SURVIVAL (85°C, 10 minutes) Negative.

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

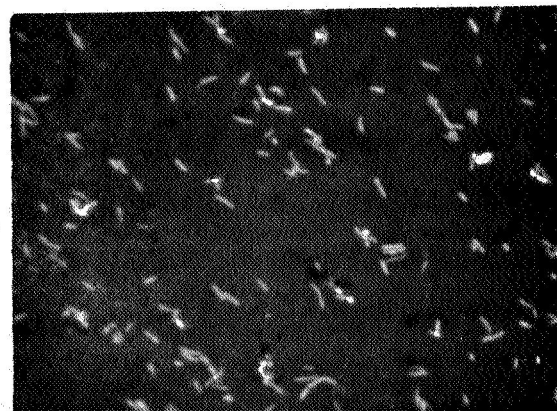
Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —

range —

24 hr.



1000X

MOTILITY: age 24 hr. Positive.

Flagella:

OTHER STAINS:

Acid fast:

Capsule:

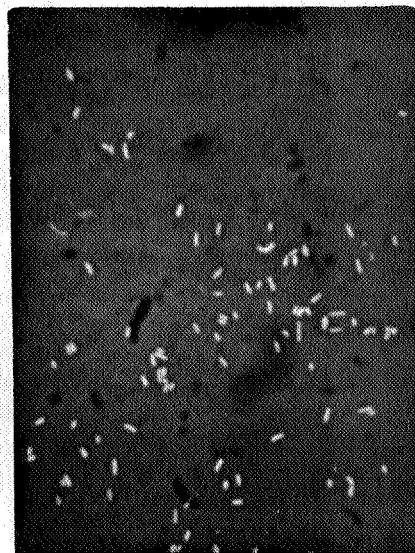
Glycogen:

Crystalline dextrans:

Fat globules:

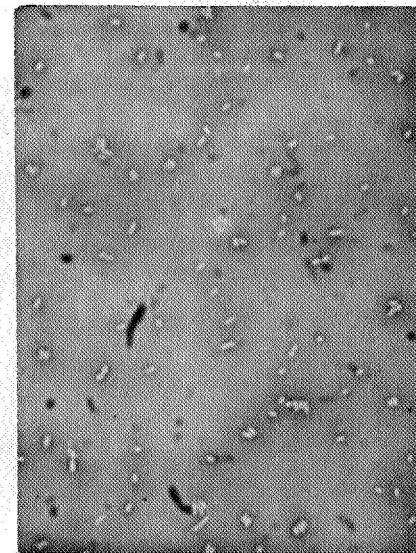
Metachromatic granules:

*Substrate mycelia present.
Wet mount (1000X) shows un-
septate highly branched
cells. Young colonies are
smooth becoming folded with
age.



1000X

48 hr.



1000X

6 da.

(46)

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 13 da.Amount of growth: abundant, moderate, scant.Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.AGAR COLONIES: age 24 da.

Macroscopic

Size: 1 mm.Shape: filamentous, irregular, oval, puntiform, round.Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.Topography: contoured, rough, smooth, striated, wrinkled.Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.Appearance by transmitted light: opaque, translucent, transparent.Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar Brite Yellow 1 1/2 la~~nonchromogenic~~ Color varies with age:- 4 da.: 2ic Honey Gold.- 30 da.: 1 1/2 pc Gold.

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.Surface growth: none, flocculent, membranous, pellicle, ring.Subsurface growth: none, granular, turbid.Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB: age 49 da.Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: Slight growth.Fat agar: Growth.Glucose-nitrate agar: Slight growth. 12 da.Slight growth on nutrient agar; no growth on glucose-nutrient agar.Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.CATALASE: positive, negative.TEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +, 45°C. +, 55°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative.Sucrose: positive, negative.Xylose: positive, negative.Citrate: positive, negative.NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative.10 da.15 da.

REDUCTIONS:

Nitrate: NO₃⁻ +, NH₄⁺ _____, gas _____, negative.Methylene blue: positive, negative.Selenite: positive, negative. slightTellurite: positive, negative.5 da.16 da.11 da.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid, alkaline, neutral, gas.Sucrose: acid, alkaline, neutral, gas.Lactose: acid, alkaline, neutral, gas.Xylose: acid, alkaline, neutral, gas.Mannitol: acid, alkaline, neutral, gas.- Fermentative: Anaerogenic.

HYDROLYSIS:

Gelatin: positive, negative.Casein: positive, negative.Fat: positive, negative.Starch: positive, negative.Cellulose: positive, negative.Urea: positive, negative.6 da.31 da.14 da.3 da.11 da.14 da.

TOLERANCES:

Salt: 2% positive, negative.7% positive, negative.10% positive, negative.pH: acid, alkaline.6 da.9 da.14 da.

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral. slight.Curd: acid, alkaline, absent, gas.Peptonization: positive, negative.Reduction: positive, negative.42 da.89 da.89 da.7 da.

OTHER REACTIONS:

H₂S from _____: positive, negative.NH₄⁺ from peptone: positive, negative.Acetylmethylcarbinol: positive, negative.Indol: positive, negative.Methyl red: positive, negative.12 da.12 da.

Descriptive Chart

<u>274b-2</u> (code number)	<u>(#6-20d) Trypticase Soy Agar</u> (medium)	<u>Chile Atacama Desert</u> (source)
<u>Nocardia sp.</u> (name of organism)	<u>28°C.</u> (temperature)	<u>W.B. Bollen & K.M. Kemper</u> (studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round & pointed.

filaments, cocci, spirals,

branching _____

Size: average —

range —

Irregular forms:

NIGROSIN STAIN:

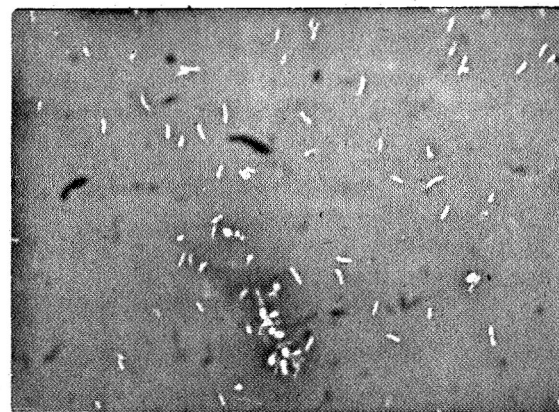
GRAM REACTION: Positive.

18 hrs:

24 hrs:

48 hrs:

24 hr.



1000X

PASTEURIZATION SURVIVAL (85°C, 10 minutes): Negative.

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —

range —

SEE PHOTOMICROGRAPHS FOR

MOTILITY: age 24 hr. Positive.

Flagella:

275a-1.

OTHER STAINS:

Acid fast:

Capsule:

Glycogen:

Crystalline dextrins:

Fat globules:

Metachromatic granules:

(46-20d)

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 16 da.Amount of growth: abundant, moderate, scant.Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.AGAR COLONIES: age 20 da.

Macroscopic

Size: 3 mm.Shape: filamentous, irregular, oval, puntiform, round.Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.Topography: contoured, rough, smooth, striated, wrinkled.Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.Appearance by transmitted light: opaque, translucent, transparent.Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar Brite Yellow 1 1/2 la~~medium~~ Color varies with age:- 4 da.: 21c Honey Gold.- 30 da.: 1 1/2c Gold.

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.Surface growth: none, flocculent, membranous, pellicle, ring.Subsurface growth: none, granular, turbid.Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB: age 86 da.Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: Moderate growth.Fat agar: Growth.Glucose-nitrate agar: No growth: 12 da.Better growth on nutrient agar; no growth on glucose-nutrient agar.Anaerobic nitrate broth: no gas: 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.CATALASE: positive, negative.TEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +, 45°C. +, 55°C. -, 5°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative.Sucrose: positive, negative.Xylose: positive, negative.Citrate: positive, negative. 13 da.NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative. 15 da.

REDUCTIONS:

Nitrate: NO₃⁻ +, NH₄⁺ _____, gas _____, negative. 5 da.Methylene blue: positive, negative.Selenite: positive, negative. slight. 16 da.Tellurite: positive, negative. 11 da.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid, alkaline _____, neutral, gas. _____Sucrose: acid _____, alkaline _____, neutral, gas. _____Lactose: acid _____, alkaline _____, neutral, gas. _____Xylose: acid _____, alkaline _____, neutral, gas. _____Mannitol: acid _____, alkaline, neutral, gas. _____Fermentative: Anaerogenic.

HYDROLYSIS:

Gelatin: positive, negative. 6 da.Casein: positive, negative. 31 da.Fat: positive, negative. 14 da.Starch: positive, negative. 3 da.Cellulose: positive, negative. 111 da.Urea: positive, negative. (no growth) 11 da.

TOLERANCES:

Salt: 2% positive, negative. 6 da.7% positive, negative. 11 da.10% positive, negative. 11 da.pH: acid _____, alkaline _____.

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral. slight. 89 da.Curd: acid, alkaline, absent, gas. 89 da.Peptonization: positive, negative. 89 da.Reduction: positive, negative. 7 da.

OTHER REACTIONS:

H₂S from _____: positive, negative. _____NH₄⁺ from peptone: positive, negative. _____Acetylmethylcarbinol: positive, negative. 12 da.Indol: positive, negative. 12 da.Methyl red: positive, negative. _____

Descriptive Chart

274b-1 (#6-20d)

(code number)

Trypticase Soy Agar

(medium)

Chile Atacama Desert

(source)

Nocardia sp.

(name of organism)

28°C.

(temperature)

W.B. Bollen & K.M. Kemper

(studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round & pointed

filaments, cocci, spirals,

branching

Size: average —

range —

Irregular forms:

NIGROSIN STAIN:



1000X

GRAM REACTION: **Positive.**

18 hrs:

24 hrs:

48 hrs:

PASTEURIZATION SURVIVAL (85°C, 10 minutes) **Negative.**

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —

range —

MOTILITY: age 24 hr. **Positive.**

Flagella:

OTHER STAINS:

Acid fast:

Capsule:

Glycogen:

Crystalline dextrans:

Fat globules:

Metachromatic granules:

II. CULTURAL CHARACTERISTICS

(#6-20d)

AGAR STROKE: age 7 da.Amount of growth: abundant, moderate, scant.Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.AGAR COLONIES: age 16 da.

Macroscopic

Size: 3 mm.Shape: filamentous, irregular, oval, puntiform, round.Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.Topography: contoured, rough, smooth, striated, wrinkled.Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistering, iridescent, opalescent.Appearance by transmitted light: opaque, translucent, transparent.Medium: blackened, blue, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar Mustard Gold 2 pe~~various~~ Color varies with age:- 4 da.: 2ic Honey Gold- 30 da.: 12pc Gold.

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.Surface growth: none, flocculent, membranous, pellicle, ring.Subsurface growth: none, granular, turbid.Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB: age 86 da.Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar Abundant growth.Fat agar: Growth.Glucose-nitrate agar: Slight growth. 12 da.Better growth on nutrient agar than glucose-nutrient agar. (No growth glucose-nutrient agar).Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.CATALASE: positive, negative.TEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 30°C. +, 37°C. +, 45°C. +, 55°C. -, 5°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative.Sucrose: positive, negative.Xylose: positive, negative.Citrate: positive, negative.NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative. 10 da. 15 da.

REDUCTIONS:

Nitrate: NO₂⁻ +, NH₄⁺ _____, gas _____, negative. 5 da.Methylene blue: positive, negative.Selenite: positive, negative. slight. 16 da.Tellurite: positive, negative. 11 da.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid, alkaline, neutral, gas.Sucrose: acid, alkaline, neutral, gas.Lactose: acid, alkaline, neutral, gas.Xylose: acid, alkaline, neutral, gas.Mannitol: acid, alkaline, neutral, gas.Fermentative: anaerogenic.

HYDROLYSIS:

Gelatin: positive, negative.Casein: positive, negative.Fat: positive, negative.Starch: positive, negative.Cellulose: positive, negative.Urea: positive, negative. (no growth)

TOLERANCES:

Salt: 2%—positive, negative.7%—positive, negative.10%—positive, negative.pH: acid _____, alkaline _____.

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral. slight.Curd: acid, alkaline, absent, gas.Peptonization: positive, negative.Reduction: positive, negative. slight

OTHER REACTIONS:

H₂S from _____: positive, negative.NH₄⁺ from peptone: positive, negative.Acetylmethylcarbinol: positive, negative.Indol: positive, negative.Methyl red: positive, negative.

Descriptive Chart

275b (#6-20d)

(code number)

Trypticase Soy Agar

(medium)

Chile Atacama Desert

(source)

Nocardia sp.

(name of organism)

28°C.

(temperature)

W.B. Bollen & K.M. Kemper

(studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round and pointed.

filaments, cocci, spirals,

branching _____

Size: average —

range —

Irregular forms:

GRAM REACTION: **Positive.**

18 hrs:

24 hrs:

48 hrs:

NIGROSIN STAIN:

24 hr.



1000X

PASTEURIZATION SURVIVAL (85°C, 10 minutes): **Negative.**

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —

range —

NIGROSIN STAIN:

8 da.



1000X

MOTILITY: age _____ **Negative.**

Flagella:

OTHER STAINS:

Acid fast:

Capsule:

Glycogen:

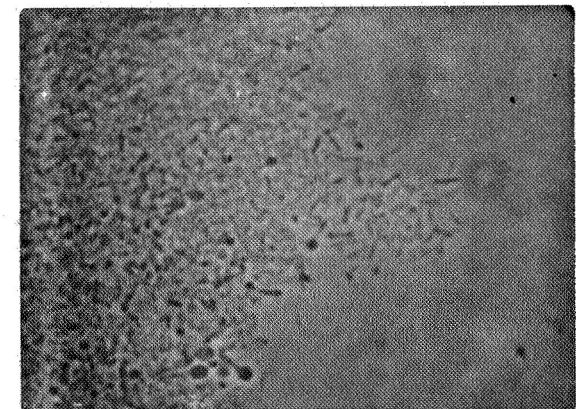
Crystalline dextrans:

Fat globules:

Metachromatic granules:

PHASE CONTRAST
OF CELLS ON
AGAR:

5 da.



1000X

(#6-20d)

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 28 da.Amount of growth: abundant, moderate, scant.Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.AGAR COLONIES: age 30 da.

Macroscopic

Size: 2 mm.Shape: filamentous, irregular, oval, puntiform, round.Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.Topography: contoured, rough, smooth, striated, wrinkled.Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.Appearance by transmitted light: opaque, translucent, transparent.Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar Lt Amber 3 ic~~XXXXXXXX~~ Color varies with age:- 4 da.: 2gc Bamboo- 30 da.: 1 1/2 ea Lt Yellow

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.Surface growth: none, flocculent, membranous, pellicle, ring.Subsurface growth: none, granular, turbid.Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB: age 86 da.Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.Rate: fast, moderate, slow.Line of puncture: filiform.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: Moderate growth.Fat agar: Growth.Glucose-nitrate agar: No growth. 12 da.Nutrient agar growth better (no growth
- on glucose-nutrient agar).Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, microaerophilic.CATALASE: positive, negative, slightTEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +,
45°C. +, 55°C. -, 5°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative.Sucrose: positive, negative.Xylose: positive, negative.Citrate: positive, negative.11 da.NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative.14 da.

REDUCTIONS:

Nitrate: NO₃⁻ +, NH₄⁺ _____, gas _____, negative _____5 da.Methylene blue: positive, negative.Selenite: positive, negative, slight.16 da.Tellurite: positive, negative.11 da.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid, alkaline, neutral, gas.Sucrose: acid, alkaline, neutral, gas.Lactose: acid, alkaline, neutral, gas.Xylose: acid, alkaline, neutral, gas.Mannitol: acid, alkaline, neutral, gas.- Fermentative: anaerogenic.

HYDROLYSIS:

Gelatin: positive, negative.6 da.Casein: positive, negative.30 da.Fat: positive, negative.14 da.Starch: positive, negative.3 da.Cellulose: positive, negative.11 da.Urea: positive, negative.11 da.

TOLERANCES:

Salt: 2% positive, negative.1 da.7% positive, negative.6 da.10% positive, negative.21 da.pH: acid, alkaline.

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral.42 da.Curd: acid, alkaline, absent, gas.89 da.Peptonization: positive, negative.89 da.Reduction: positive, negative, slight.7 da.

OTHER REACTIONS:

H₂S from _____: positive, negative.NH₄⁺ from peptone: positive, negative.Acetylmethylcarbinol: positive, negative.12 da.Indol: positive, negative.12 da.Methyl red: positive, negative.

Descriptive Chart

248b (#25-6) Trypticase Soy Agar Chile Atacama Desert
(code number) (medium) (source)

Mycobacterium sp.* 28°C. W.B. Bollen & K.M. Kemper
(name of organism) (temperature) (studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round & pointed.

filaments, cocci, spirals,

branching _____

Size: average —

range — See Dichotomous Key p. 16.

Irregular forms:

NIGROSIN STAIN:

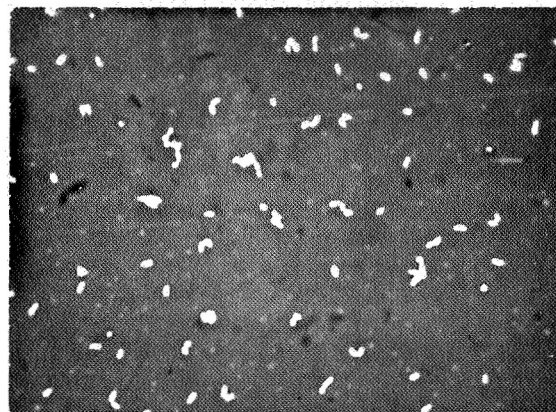
GRAM REACTION: Positive.

18 hrs:

24 hr.

24 hrs:

48 hrs:



1000X

PASTEURIZATION SURVIVAL (85°C, 10 minutes): Negative.

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —

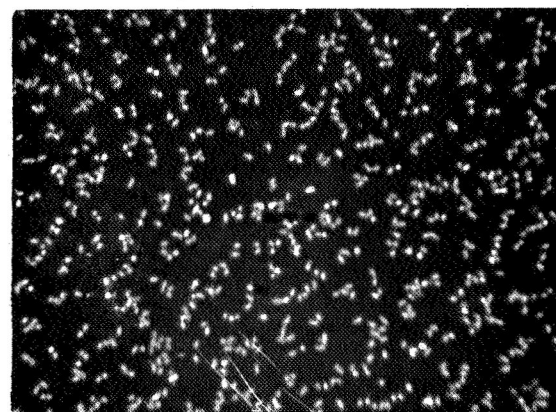
range —

NIGROSIN STAIN:

MOTILITY: age 1 da. Negative.

17 da.

Flagella:



1000X

OTHER STAINS:

Acid fast:

Capsule:

Glycogen:

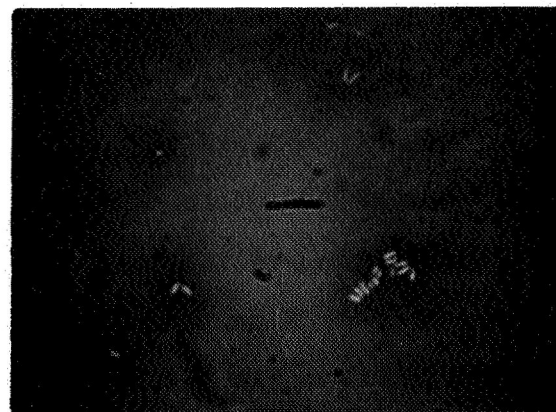
Crystalline dextrins:

Fat globules:

Metachromatic granules:

NIGROSIN STAIN:

18 hr.



1000X

*Identity of this isolant resembles
Mycobacterium brevicale Krasil'nikov 1941 or
M. rubropertinctum (Hefferan) Ford 1927 (Jensen,
1934).

(#25-6)

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 13 da.Amount of growth: abundant, moderate, scant.Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.AGAR COLONIES: age 13 da.

Macroscopic

Size: 4 mm.Shape: filamentous, irregular, oval, puntiform, round.Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.Topography: contoured, rough, smooth, striated, wrinkled.Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.Appearance by transmitted light: opaque, translucent, transparent.Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar Bittersweet 5 pcPotato slant (Burnt Orange)

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.Surface growth: none, flocculent, membranous, pellicle, ring.Subsurface growth: none, granular, turbid.Sediment: none, compact, flaky, flocculent, granular, viscid.Odor: resembling _____GELATIN STAB: age 73 da.Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: Moderate growth.Fat agar: Growth.Glucose-nitrate agar: Growth. 12 da.Growth nutrient agar; better growth on glucose-nutrient agar.Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.CATALASE: positive, negative.TEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +, 45°C. -, 55°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative.Sucrose: positive, negative.Xylose: positive, negative.Citrate: positive, negative.10 da.NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative.5 da.

REDUCTIONS:

Nitrate: NO₂⁻ +, NH₄⁺ _____, gas _____, negative. 5 da.Methylene blue: positive, negative.34 da.Selenite: positive, negative.4 da.Tellurite: positive, negative.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid, alkaline, neutral, gas.Sucrose: acid, alkaline, neutral, gas.Lactose: acid, alkaline, neutral, gas.Xylose: acid, alkaline, neutral, gas.Mannitol: acid, alkaline, neutral, gas.

HYDROLYSIS:

Gelatin: positive, negative.14 da.Casein: positive, negative.34 da.Fat: positive, negative.14 da.Starch: positive, negative. slight.11 da.Cellulose: positive, negative.111 da.Urea: positive, negative.4 da.

TOLERANCES:

Salt: 2% positive, negative.6 da.7% positive, negative.6 da.10% positive, negative.9 da.pH: acid, alkaline.

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral.12 da.Curd: acid, alkaline, absent, gas.89 da.Peptonization: positive, negative.89 da.Reduction: positive, negative. slight.7 da.Growth lt. orange in bottom of tube.

OTHER REACTIONS:

H₂S from _____: positive, negative.NH₄⁺ from peptone: positive, negative.Acetylmethylcarbinol: positive, negative.12 da.Indol: positive, negative.12 da.Methyl red: positive, negative.

Descriptive Chart

248Aa-1 (#25/TSA)
(code number)

Trypticase Soy Agar
(medium)

Chile Atacama Desert
(source)

Mycobacterium sp.*
(name of organism)

28°C.
(temperature)

W.B. Bollen & K.M. Kemper
(studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round & pointed.
filaments, cocci, spirals,
branching

Size: average —

range — See Dichotomous Key p. 16.

Irregular forms:

NIGROSIN STAIN:

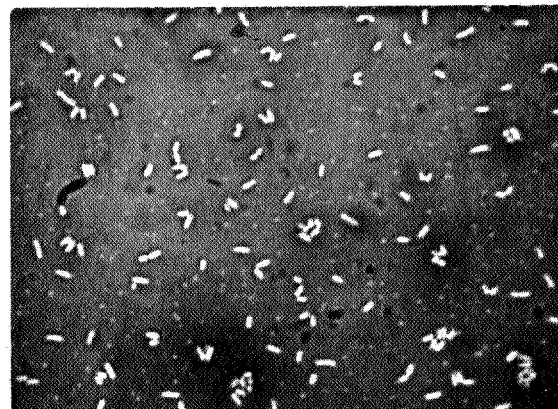
GRAM REACTION: Positive.

18 hrs:

24 hr.

24 hrs:

48 hrs:



1000X

PASTEURIZATION SURVIVAL (85°C, 10 minutes): Negative.

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —

range —

MOTILITY: age 1 da. Negative.

Flagella:

NIGROSIN STAIN:

OTHER STAINS:

24 hr.

Acid fast:

Capsule:

Glycogen:

Crystalline dextrans:

Fat globules:

Metachromatic granules:



1000X

*Identity of this isolant resembles Mycobacterium brevicale Krasil'nikov 1941 or
M. rubropertinctum (Hefferan) Ford 1927 (Jensen, 1934).

II. CULTURAL CHARACTERISTICS

(#25/TSA)

AGAR STROKE: age 16 da.Amount of growth: abundant, moderate, scant.Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.AGAR COLONIES: age 23 da.

Macroscopic

Size: 3 mm.Shape: filamentous, irregular, oval, puntiform, round.Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.Topography: contoured, rough, smooth, striated, wrinkled.Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.Appearance by transmitted light: opaque, translucent, transparent.Medium: blackened, blue, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color)

Trypticase soy agar Coral (CHM No.) 6 lc

Potato slant

—

—

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.Surface growth: none, flocculent, membranous, pellicle, ring.Subsurface growth: none, granular, turbid.Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB: age 73 da.Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: Abundant growth.Fat agar: Growth.Glucose-nitrate agar: No growth. 12 da.Growth on glucose-nutrient agar better than on nutrient agar.Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.CATALASE: positive, negative.TEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +, 45°C. -, 55°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative.Sucrose: positive, negative.Xylose: positive, negative.Citrate: positive, negative. 13 da.NH₄⁺ AS SOLE NITROGEN SOURCE: positive. 5 da.
negative.

REDUCTIONS:

Nitrate: NO₂⁻ +, NH₄⁺ _____, gas _____, negative. 5 da.Methylene blue: positive, negative.Selenite: positive, negative. 34 da.Tellurite: positive, negative. 4 da.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid _____, alkaline _____, neutral, gas. _____Sucrose: acid _____, alkaline _____, neutral, gas. _____Lactose: acid _____, alkaline _____, neutral, gas. _____Xylose: acid _____, alkaline _____, neutral, gas. _____Mannitol: acid _____, alkaline _____, neutral, gas. _____

HYDROLYSIS:

Gelatin: positive, negative. 14 da.Casein: positive, negative. 31 da.Fat: positive, negative. 14 da.Starch: positive, negative. slight. 11 da.Cellulose: positive, negative. 60 da.Urea: positive, negative. slight. 11 da.

TOLERANCES:

Salt: 2% positive, negative. 6 da.7% positive, negative. 6 da.10% positive, negative. 9 da.pH: acid _____, alkaline _____

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral. 12 da.Curd: acid, alkaline, absent. gas. 89 da.Peptonization: positive, negative. 89 da.Reduction: positive, negative. slight. 11 da.

OTHER REACTIONS:

H₂S from _____: positive, negative.NH₄⁺ from peptone: positive, negative.Acetylmethylcarbinol: positive, negative. 12 da.Indol: positive, negative. 12 da.Methyl red: positive, negative.

Descriptive Chart

248Aa-2 (#25/TSA)
(code number)

Trypticase Soy Agar
(medium)

Chile Atacama Desert
(source)

Mycobacterium sp.*
(name of organism)

28°C.
(temperature)

W.B. Bollen & K.M. Kemper
(studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: ~~rods~~, ends round & pointed,
filaments, cocci, spirals,
branching _____

Size: average —
range — See Dichotomous Key p. 16.

Irregular forms:

NIGROSIN STAIN:

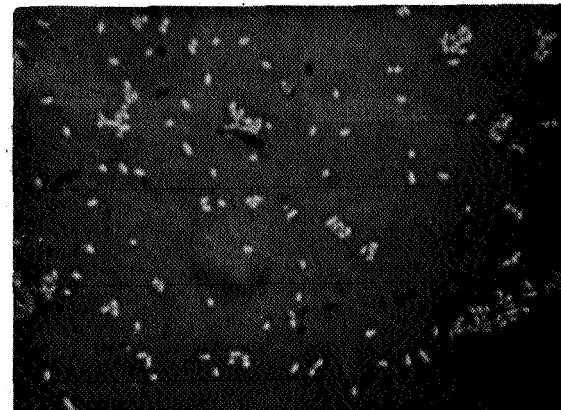
GRAM REACTION: **Positive.**

18 hrs:

24 hr.

24 hrs:

48 hrs:



1000X

PASTEURIZATION SURVIVAL (85°C, 10 minutes) **Negative.**

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —

range —

MOTILITY: age 1 da.

Flagella:

NIGROSIN STAIN:

OTHER STAINS:

Acid fast:

Capsule:

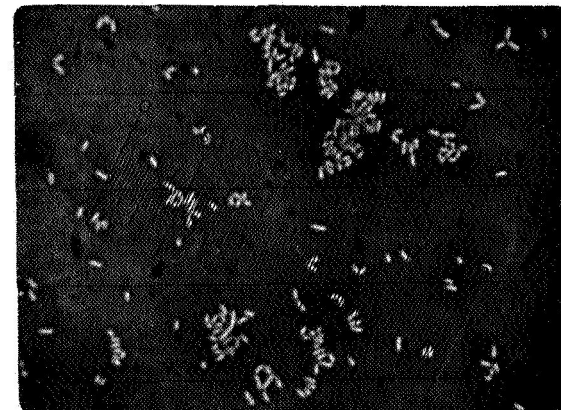
Glycogen:

Crystalline dextrans:

Fat globules:

Metachromatic granules:

24 hr.



1000X

*Identity of this isolant resembles Mycobacterium brevicale Krasil'nikov or

M. rubropertinctum (Hefferan) Ford 1927 (Jensen, 1934).

II. CULTURAL CHARACTERISTICS

(#25/TSA)

AGAR STROKE: age 16 da.
 Amount of growth: abundant, moderate, scant.
 Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.
 Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.

AGAR COLONIES: age 23 da.
 Macroscopic
 Size: 0.5 mm.
 Shape: filamentous, irregular, oval, puntiform, round.
 Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.
 Topography: contoured, rough, smooth, striated, wrinkled.
 Habit: compact, spreading.
 Microscopic (100x)
 Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.
 Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.
 Optical properties
 Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.
 Appearance by transmitted light: opaque, translucent, transparent.
 Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.
 Chromogenesis:
 (medium) (color) (CHM No.)
 Trypticase soy agar Lt. Lacquer Red 6 nc
 Potato slant
 —
 —

NUTRIENT BROTH: age _____
 Amount of growth: abundant, moderate, scant.
 Surface growth: none, flocculent, membranous, pellicle, ring.
 Subsurface growth: none, granular, turbid.
 Sediment: none, compact, flaky, flocculent, granular, viscid.
 Odor: resembling _____

GELATIN STAB: age 73 da.
 Liquefaction: none, crateriform, infundibuliform, napiform, saecate, stratiform.
 Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:
 Soybean infusion agar Abundant growth.
 Fat agar: Growth.
 Glucose-nitrate agar: No growth. 12 da.
Growth on glucose-nutrient agar better than growth on nutrient agar.

Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____
 G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.

CATALASE: positive, negative.

TEMPERATURE RELATIONSHIPS: age 17 da.
 Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +,
 45°C. —, 55°C. —, 5°C. —.

SOLE CARBON SOURCE: age _____
 Glucose: positive, negative.
 Sucrose: positive, negative.
 Xylose: positive, negative.
 Citrate: positive, negative. 13 da.
 NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative. 5 da.

REDUCTIONS:
 Nitrate: NO₂⁻ +, NH₄⁺ —, gas —, negative. 5 da.
 Methylene blue: positive, negative.
 Selenite: positive, negative. 34 da.
 Tellurite: positive, negative. 4 da.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid, alkaline —, neutral, gas —^c
 Sucrose: acid, alkaline —, neutral, gas. —
 Lactose: acid, alkaline —, neutral, gas. —
 Xylose: acid, alkaline —, neutral, gas. —
 Mannitol: acid, alkaline —, neutral, gas. —

HYDROLYSIS:

Gelatin: positive, negative. 14 da.
 Casein: positive, negative. 24 da.
 Fat: positive, negative. 14 da.
 Starch: positive, negative, slight. 11 da.
 Cellulose: positive, negative. 60 da.
 Urea: positive, negative, slight. 11 da.

TOLERANCES:

Salt: 2% positive, negative. 6 da.
 7% positive, negative. 6 da.
 10% positive, negative. 6 da.
 pH: acid, alkaline —

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral. 12 da.
 Curd: acid, alkaline, absent, gas. 89 da.
 Peptonization: positive, negative. 89 da.
 Reduction: positive, negative. 89 da.
Growth lt. orange in bottom of tube.

OTHER REACTIONS:

H₂S from _____: positive, negative.
 NH₄⁺ from peptone: positive, negative.
 Acetylmethylcarbinol: positive, negative. 12 da.
 Indol: positive, negative. 12 da.
 Methyl red: positive, negative.

Descriptive Chart

247Eb (#24)

(code number)

Trypticase Soy Agar

(medium)

Chile Atacama Desert

(source)

Arthrobacter sp.

(name of organism)

28°C.

(temperature)

W.B. Bollen & K.M. Kemper

(studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round & pointed.
filaments, cocci, spirals,
branching

Size: average —
range —

Irregular forms:

GRAM REACTION Positive.

18 hrs:

24 hrs:

48 hrs:

NIGROSIN STAIN:

TSA 24 hr.



1000X

PASTEURIZATION SURVIVAL (85°C, 10 minutes): Negative

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

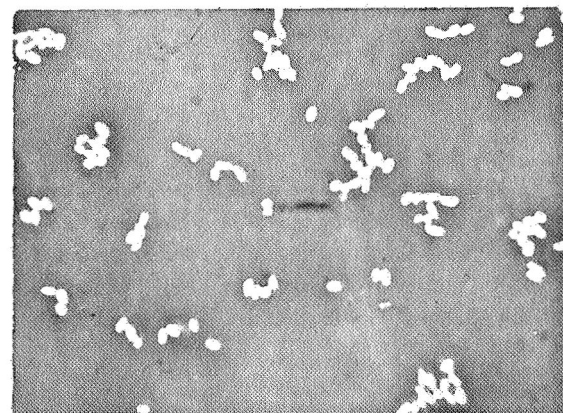
Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —
range —

NIGROSIN STAIN:
GLUCOSE-NITRATE AGAR:



1000X

MOTILITY: age 1 da. Negative.

Flagella:

24 hr.

OTHER STAINS:

Acid fast:

Capsule:

Glycogen:

Crystalline dextrans:

Fat globules:

Metachromatic granules:

NIGROSIN STAIN:
AMMONIUM UTILIZATION AGAR:

24 hr.



1000X

II. CULTURAL CHARACTERISTICS

(#24)

AGAR STROKE: age 16 da.Amount of growth: abundant, moderate, scant.Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.Consistency: adherent, brittle, butyrus, membranous, moist, slimy, soft, tough, viscid, waxy.AGAR COLONIES: age 16 da.

Macroscopic

Size: 2 mm.Shape: filamentous, irregular, oval, puntiform, round.Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.Topography: contoured, rough, smooth, striated, wrinkled.Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.Appearance by transmitted light: opaque, translucent, transparent.Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color)

(CHM No.)

Trypticase soy agar Lt Ivory 2 ca

Potato slant

—
—

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.Surface growth: none, flocculent, membranous, pellicle, ring.Subsurface growth: none, granular, turbid.Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB: age 14 da.Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar Abundant growth.Fat agar: Growth.Glucose-nitrate agar: Abundant growth. 1 da.Growth on glucose-nutrient agar more abundant than on nutrient agar (both abundant).

DNA

Anaerobic nitrate broth: no gas. 14 da.Glucose-asparagine agar: Abundant growth.

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.CATALASE: positive, negative.

TEMPERATURE RELATIONSHIPS: age _____

Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +, 45°C. -, 55°C. -. 5°C. +.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative.Sucrose: positive, negative.Xylose: positive, negative.Citrate: positive, negative. acid 1 da.NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative. 1 da.

REDUCTIONS:

Nitrate: NO₃⁻ _____, NH₄⁺ _____, gas _____, negative. 2 da. ?*Methylene blue: positive, negative.Selenite: positive, negative. 46 da.Tellurite: positive, negative. 15 da.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid, alkaline _____, neutral, gas. _____Sucrose: acid _____, alkaline _____, neutral, gas. _____Lactose: acid _____, alkaline _____, neutral, gas. _____Xylose: acid _____, alkaline _____, neutral, gas. _____Mannitol: acid _____, alkaline, neutral, gas. _____

HYDROLYSIS:

Gelatin: positive, negative. 14 da.Casein: positive, negative. 4 da.Fat: positive, negative. 48 da.Starch: positive, negative, slight. 6 da.Cellulose: positive, negative. 30 da.Urea: positive, negative. 2 da.

TOLERANCES:

Salt: 2%—positive, negative. 2 da.7%—positive, negative. 14 da.10%—positive, negative. 14 da.pH: acid _____, alkaline _____.

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral. 14 da.Curd: acid, alkaline, absent, gas. 30 da.Peptonization: positive, negative. 30 da.Reduction: positive, negative. slight. 14 da.

OTHER REACTIONS:

H₂S from _____: positive, negative. _____NH₄⁺ from peptone: positive, negative. _____Acetylmethylcarbinol: positive, negative. 14 da.Indol: positive, negative. 14 da.Methyl red: positive, negative. _____

Descriptive Chart

274a (code number)	(#6-20d)	Trypticase Soy Agar (medium)	Chile Atacama Desert (source)
<u>Mycococcus capsulatus</u> *	28°C.		W.B. Bollen & K.M. Kemper
(name of organism)	(temperature)		(studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round,
filaments, cocci, spirals,
branching

Size: average — Length 24 hr. cells average 1.2 μ .
range — Diameter 72 hr. cells 0.74-1.25 μ .

Irregular forms: Resting cells 2.2 μ diameter.

Coccoid. Majority of cells 0.8 μ diameter.

GRAM REACTION: Positive.

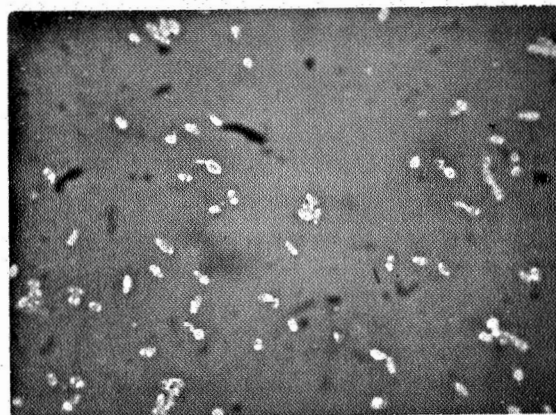
18 hrs:

24 hrs:

48 hrs:

NIGROSIN STAIN:

72 hr.



1000X

PASTEURIZATION SURVIVAL (85°C, 10 minutes): Negative.

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

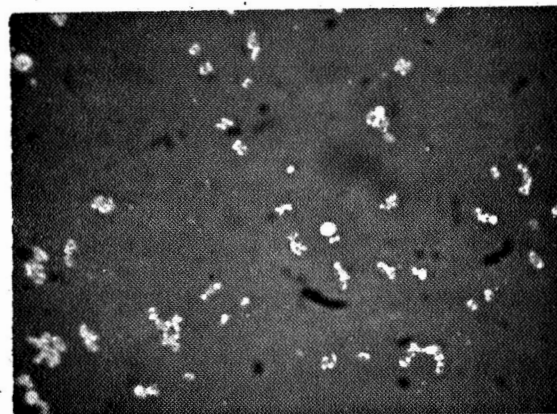
Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —

range —

NIGROSIN STAIN:

72 hr.



1000X

MOTILITY: age 1 da. Negative.

Flagella:

OTHER STAINS:

Acid fast:

Capsule:

Glycogen:

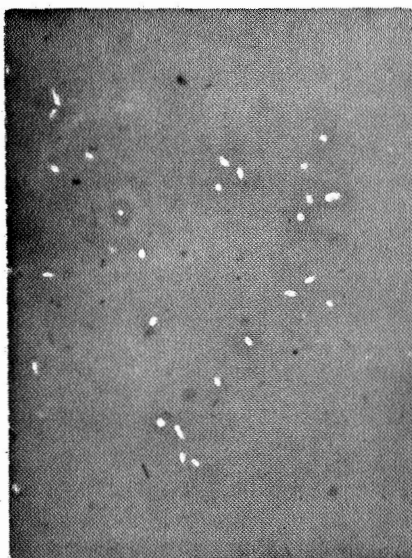
Crystalline dextrans:

Fat globules:

Metachromatic granules:

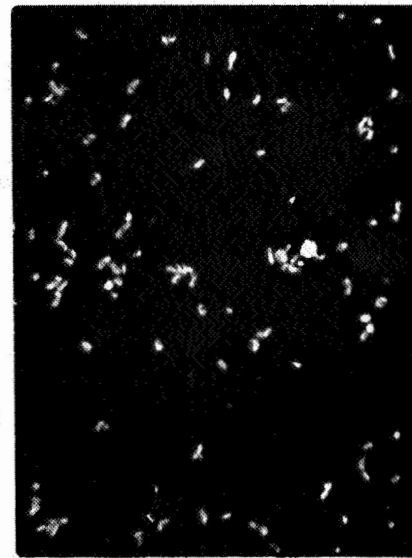
NIGROSIN STAINS:

24 hr. & 22 da.



1000X

24 hr.



1000X

22 da.

*(Krasil'nikov. 1941. 127.)

II. CULTURAL CHARACTERISTICS

(46-20d)

AGAR STROKE: age 10 da.
 Amount of growth: *abundant, moderate, scant.*
 Form: *aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.*
 Consistency: *adherent, brittle, buturous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 20 da.

Macroscopic

Size: 0.5 mm.

Shape: *filamentous, irregular, oval, punctiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

(medium)

(color)

(CHM No.)

Trypticase soy agar **Pastel Orange** 41c

Potato slant

—
—

NUTRIENT BROTH: age _____

Amount of growth: *abundant, moderate, scant.*

Surface growth: *none, flocculent, membranous, pellicle, ring.*

Subsurface growth: *none, granular, turbid.*

Sediment: *none, compact, flaky, flocculent, granular, viscid.*

Odor: *resembling _____.*

GELATIN STAB: age 73 da.

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: **Moderate growth.**

Fat agar: **Growth (much better than on TSA).**

Glucose-nitrate agar: **No growth. 14 da.**

Growth on nutrient agar; no growth on glucose-nutrient agar.

Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: *aerobic, anaerobic, facultative, micro-aerophilic.*

CATALASE: *positive, negative.* **slight.**

TEMPERATURE RELATIONSHIPS: age 7 da.

Growth at 10°C. —, 20°C. +, 30°C. +, 40°C. +, 45°C. —, 55°C. —. **15°C. + slight.**

SOLE CARBON SOURCE: age _____

Glucose: *positive, negative.*

Sucrose: *positive, negative.*

Xylose: *positive, negative.*

Citrate: *positive, negative.* **slight. 11 da.**

NH₄⁺ AS SOLE NITROGEN SOURCE: *positive, negative.* **14 da.**

REDUCTIONS:

very slight.
 Nitrate: NO₂⁻ +, NH₄⁺ —, gas —, negative. **12 da.**

Methylene blue: *positive, negative.*

Selenite: *positive, negative.* **34 da.**

Tellurite: *positive, negative.* **11 da.**

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: *acid, alkaline, neutral, gas.*

Sucrose: *acid, alkaline, neutral, gas.*

Lactose: *acid, alkaline, neutral, gas.*

Xylose: *acid, alkaline, neutral, gas.*

Mannitol: *acid, alkaline, neutral, gas.*

HYDROLYSIS:

Gelatin: *positive, negative.* **16 da.**

Casein: *positive, negative.* **31 da.**

Fat: *positive, negative.* **14 da.**

Starch: *positive, negative.* **slight. 11 da.**

Cellulose: *positive, negative.* **111 da.**

Urea: *positive, negative.* **(no growth) 11 da.**

TOLERANCES:

Salt: 2%—*positive, negative.* **6 da.**

7%—*positive, negative.* **21 da.**

10%—*positive, negative.* **21 da.**

pH: *acid, alkaline.*

LITMUS MILK REACTIONS:

Reaction: *acid, alkaline, neutral.* **89 da.**

Curd: *acid, alkaline, absent, gas.* **89 da.**

Peptonization: *positive, negative.* **89 da.**

Reduction: *positive, negative.* **89 da.**

OTHER REACTIONS:

H₂S from _____: *positive, negative.*

NH₄⁺ from peptone: *positive, negative.*

Acetylmethylcarbinol: *positive, negative.* **12 da.**

Indol: *positive, negative.* **12 da.**

Methyl red: *positive, negative.*

Descriptive Chart

246a (#26/TSA)
(code number)

Trypticase Soy Agar
(medium)

Chile Atacama Desert
(source)

Bacillus cereus
(name of organism)

28°C.
(temperature)

W.B. Bollen & K.M. Kemper
(studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends square & round,
filaments, cocci, spirals,
branching _____

Size: average —
range —

Irregular forms:

NIGROSIN STAIN:

GRAM REACTION: **Positive.**

18 hrs:

24 hrs:

48 hrs:



1000X

PASTEURIZATION SURVIVAL (85°C, 10 minutes): **Positive.**

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —
range —

SPORE STAIN:

MOTILITY: age _____.

Flagella:

OTHER STAINS:

Acid fast:

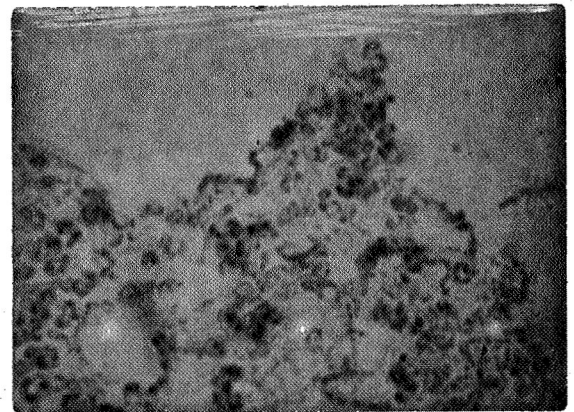
Capsule:

Glycogen:

Crystalline dextrans:

Fat globules:

Metachromatic granules:



1000X

II. CULTURAL CHARACTERISTICS

(#26/TSA)

AGAR STROKE: age 22 da.Amount of growth: abundant, moderate, scant.Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.AGAR COLONIES: age 23 da.

Macroscopic

Size: 5 mm.Shape: filamentous, irregular, oval, puntiform, round.Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.Topography: contoured, rough, smooth, striated, wrinkled.Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.Appearance by transmitted light: opaque, translucent, transparent.Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color)

(CHM No.)

Trypticase soy agar lt Ivory 2 ca.

Potato slant

—

—

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.Surface growth: none, flocculent, membranous, pellicle, ring.Subsurface growth: none, granular, turbid.Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB: age _____

Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: Abundant growth.

Fat agar:

Glucose-nitrate agar: Slight growth. 7 da.Growth on glucose-nutrient agar better than on nutrient agar.Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.CATALASE: positive, negative.TEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +, 45°C. +, 55°C. -, 5°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative.Sucrose: positive, negative.Xylose: positive, negative.Citrate: positive, negative.NH₄⁺ AS SOLE NITROGEN SOURCE: positive. 2 da.Mannitol: Positive (no acid). 5 da.6 da.

REDUCTIONS:

Nitrate: NO₃⁻ _____, NH₄⁺ _____, gas _____, negative. 12 da.Methylene blue: positive, negative.Selenite: positive, negative. 4 da.Tellurite: positive, negative.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid _____, alkaline _____, neutral _____, gas _____Sucrose: acid _____, alkaline _____, neutral _____, gas _____Lactose: acid _____, alkaline _____, neutral _____, gas _____Xylose: acid _____, alkaline _____, neutral _____, gas _____Mannitol: acid _____, alkaline _____, neutral _____, gas _____

HYDROLYSIS:

Gelatin: positive, negative.Casein: positive, negative.Fat: positive, negative.Starch: positive, negative.Cellulose: positive, negative.Urea: positive, negative.

TOLERANCES:

Salt: 2%—positive, negative.7%—positive, negative.10%—positive, negative.pH: acid _____, alkaline _____

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral.Curd: acid, alkaline, absent, gas.Peptonization: positive, negative.Reduction: positive, negative.

OTHER REACTIONS:

H₂S from _____: positive, negative.NH₄⁺ from peptone: positive, negative.Acetylmethylcarbinol: positive, negative.Indol: positive, negative.Methyl red: positive, negative.

Descriptive Chart

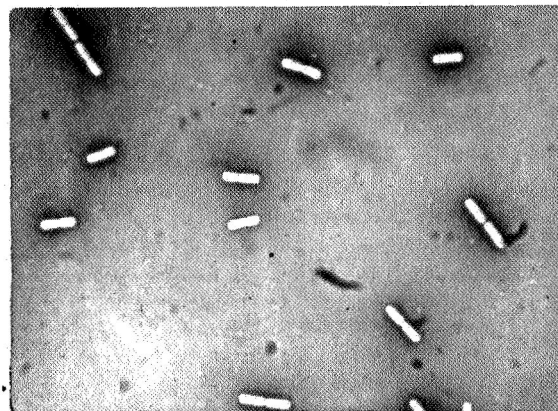
260A (code number)	(#25/TSA) Trypticase Soy Agar (medium)	Chile Atacama Desert (source)
<u>Bacillus cereus</u> (name of organism)	28°C. (temperature)	W.B. Bollen & K.M. Kemper (studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: ~~rods~~, ends square & round,
filaments, cocci, spirals,
branching _____
Size: average —
range —
Irregular forms:

NIGROSIN STAIN:



1000X

GRAM REACTION: Positive.

18 hrs:
24 hrs:
48 hrs:

PASTEURIZATION SURVIVAL (85°C, 10 minutes): Positive.

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.
Endospores: swollen, not swollen,
Position: central to excentric, terminal, subterminal.
Shape: spherical, ellipsoidal, cylindrical, oval.
size: average —
range —

SPORE STAIN:



1000X

MOTILITY: age _____.
Flagella:

OTHER STAINS:

Acid fast:
Capsule:
Glycogen:
Crystalline dextrans:
Fat globules:
Metachromatic granules:

II. CULTURAL CHARACTERISTICS

(#25/TSA)

AGAR STROKE: age 14 da.

Amount of growth: abundant, moderate, scant.

Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.

Consistency: adherent, brittle, buturous, membranous, moist, slimy, soft, tough, viscid, waxy.

AGAR COLONIES: age 14 da.

Macroscopic

Size: 3 mm.

Shape: filamentous, irregular, oval, puntiform, round.

Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.

Topography: contoured, rough, smooth, striated, wrinkled.

Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.

Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, iridescent, opalescent.

Appearance by transmitted light: opaque, translucent, transparent.

Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color)

(CHM No.)

Trypticase soy agar Lt Ivory

2 ca

Potato slant

TEST SUPPORT BY OPERATIONS TEAM

NUTRIENT BROTH:

— DSIF OVT

Amount of growth: abundant, moderate, scant.

Surface growth: none, flocculent, membranous, petticle, ring.

Subsurface growth: none, granular, turbid.

Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB:

age _____

Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.

Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: Abundant growth.

Fat agar:

Glucose-nitrate agar: Growth. 7 da.

Glucose-nutrient agar growth slightly better than nutrient agar.

Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

605-187

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.

CATALASE: positive, negative.

TEMPERATURE RELATIONSHIPS: age 7 da.

Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +,

45°C. +, 55°C. -, 50°C. -, 50°C. -.

SOLE CARBON SOURCE:

age _____

Glucose: positive, negative.

Sucrose: positive, negative.

Xylose: positive, negative.

Citrate: positive, negative.

NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative.

REDUCTIONS:

Nitrate: NO₂⁻ _____, NH₄⁺ _____, gas _____, negative. _____

Methylene blue: positive, negative.

Selenite: positive, negative.

Vitalite: positive, negative.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid, alkaline, neutral, gas. _____

Sucrose: acid, alkaline, neutral, gas. _____

Lactose: acid, alkaline, neutral, gas. _____

Xylose: acid, alkaline, neutral, gas. _____

Mannitol: acid, alkaline, neutral, gas. _____

HYDROLYSIS:

Gelatin: positive, negative.

Casein: positive, negative.

Fat: positive, negative.

Starch: positive, negative.

Cellulose: positive, negative.

Urea: positive, negative.

TOLERANCES:

Salt: 2% positive, negative.

7% positive, negative.

10% positive, negative.

pH: acid, alkaline.

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral.

Curd: acid, alkaline, absent, gas.

Peptonization: positive, negative.

Reduction: positive, negative.

OTHER REACTIONS:

H₂S from _____: positive, negative.

NH₄⁺ from peptone: positive, negative.

Acetylmethylcarbinol: positive, negative.

Indol: positive, negative.

Methyl red: positive, negative.

Descriptive Chart

260b	(#26/TSA)	Trypticase Soy Agar	Chile Atacama Desert
(code number)		(medium)	(source)
<u>Bacillus</u> sp.*	28°C.	W.B. Bollen & K.M. Kemper	
(name of organism)	(temperature)	(studied by)	

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

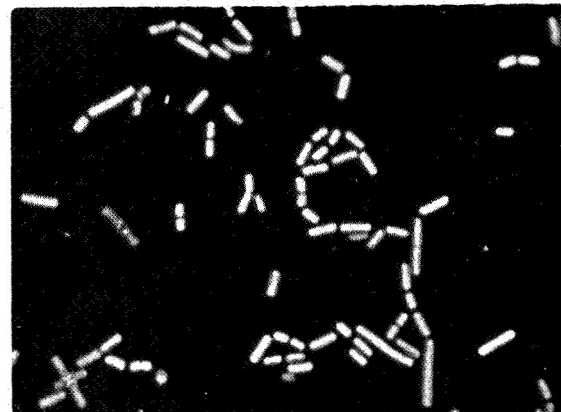
MORPHOLOGY:

Form: rods, ends square & rounded,
filaments, cocci, spirals,
branching _____

Size: average —
range —

Irregular forms:

NIGROSIN STAIN:



1000X

GRAM REACTION: Positive.

18 hrs:

24 hrs:

48 hrs:

PASTEURIZATION SURVIVAL (85°C, 10 minutes): Positive.

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

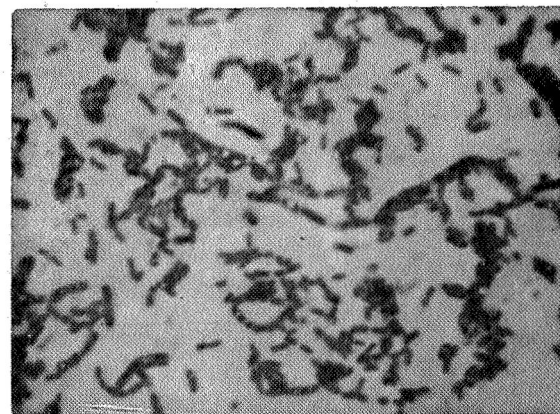
Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —
range —

SPORE STAIN:



1000X

MOTILITY: age _____.

Flagella:

OTHER STAINS:

Acid fast:

Capsule:

Glycogen:

Crystalline dextrans:

Fat globules:

Metachromatic granules:

*The cell size of this microorganism places it into Group I of the dichotomous key for genus Bacillus. The physiological reactions of this isolant are not in close accordance with either Bacillus megaterium nor B. cereus.

II. CULTURAL CHARACTERISTICS

(#26/TSA)

AGAR STROKE: age 16 da.

Amount of growth: *abundant, moderate, scant.*

Form: *aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.*

Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*

AGAR COLONIES: age 16 da.

Macroscopic

Size: 3 mm.

Shape: *filamentous, irregular, oval, puntiform, round.*

Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*

Topography: *contoured, rough, smooth, striated, wrinkled.*

Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*

Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*

Appearance by transmitted light: *opaque, translucent, transparent.*

Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar **Bisque** 3 ec

Potato slant

—
—

NUTRIENT BROTH: age _____

Amount of growth: *abundant, moderate, scant.*

Surface growth: *none, flocculent, membranous, pellicle, ring.*

Subsurface growth: *none, granular, turbid.*

Sediment: *none, compact, flaky, flocculent, granular, viscid.*

Odor: resembling _____

GELATIN STAB: age _____

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*

Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: **Abundant growth.**

Fat agar:

Glucose-nitrate agar: **No growth. 12 da.**

**Slight growth nutrient agar; no growth
— glucose-nutrient agar.**

Anaerobic nitrate broth: no gas: 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: *aerobic, anaerobic, facultative, micro-aerophilic.*

CATALASE: *positive, negative.*

TEMPERATURE RELATIONSHIPS: age 17 da.

Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +,
45°C. +, 55°C. -, 5°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: *positive, negative.*

Sucrose: *positive, negative.*

Xylose: *positive, negative.*

Citrate: *positive, negative.* 2 da.

NH₄⁺ AS SOLE NITROGEN SOURCE: *positive, negative.* 5 da.

Mannitol: Positive (no acid) 6 da.

REDUCTIONS:

Nitrate: NO₂⁻ +, NH₄⁺ _____, gas _____, negative. 5 da.

Methylene blue: *positive, negative.*

Selenite: *positive, negative. slight.* 16 da.

Tellurite: *positive, negative.*

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: *acid _____, alkaline _____, neutral, gas.*

Sucrose: *acid _____, alkaline _____, neutral, gas.*

Lactose: *acid _____, alkaline _____, neutral, gas.*

Xylose: *acid _____, alkaline _____, neutral, gas.*

Mannitol: *acid _____, alkaline, neutral, gas.*

HYDROLYSIS:

Gelatin: *positive, negative.*

Casein: *positive, negative.*

Fat: *positive, negative.*

Starch: *positive, negative.*

Cellulose: *positive, negative.*

Urea: *positive, negative.*

TOLERANCES:

Salt: 2%—*positive, negative.*

7%—*positive, negative.*

10%—*positive, negative.*

pH: *acid _____, alkaline _____.*

LITMUS MILK REACTIONS:

Reaction: *acid, alkaline, neutral.*

Curd: *acid, alkaline, absent, gas.*

Peptonization: *positive, negative.*

Reduction: *positive, negative.*

OTHER REACTIONS:

H₂S from _____: *positive, negative.*

NH₄⁺ from peptone: *positive, negative.*

Acetylmethylcarbinol: *positive, negative.*

Indol: *positive, negative.*

Methyl red: *positive, negative.*

14 da.

30 da.

3 da.

60 da.

5 da.

11 da.

11 da.

11 da.

42 da.

42 da.

42 da.

42 da.

12 da.

12 da.

Descriptive Chart

248a	(#6-20d)	Trypticase Soy Agar	Chile Atacama Desert
(code number)		(medium)	(source)
<u>Bacillus pumilus</u>	28°C.		W.B. Bollen & K.M. Kemper
(name of organism)	(temperature)		(studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods ends round,
filaments, cocci, spirals,
branching

Size: average —
range —

Irregular forms:

NIGROSIN STAIN



1000X

GRAM REACTION: **Positive.**

18 hrs:

24 hrs:

48 hrs:

PASTEURIZATION SURVIVAL (85°C, 10 minutes): **Positive.**

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

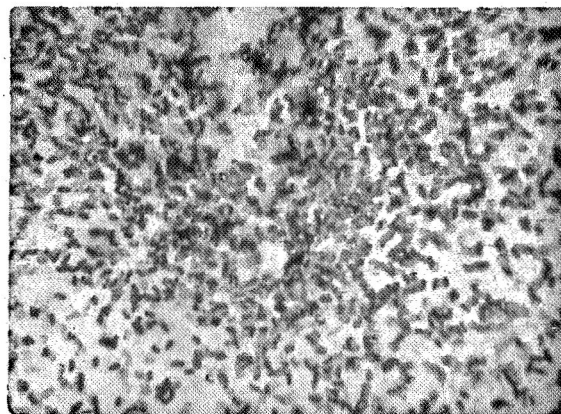
Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —
range —

SPORE STAIN



1000X

MOTILITY: age

Flagella:

OTHER STAINS:

Acid fast:

Capsule:

Glycogen:

Crystalline dextrans:

Fat globules:

Metachromatic granules:

(#6-20d)

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 14 da.Amount of growth: abundant, moderate, scant.Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.Consistency: adherent, brittle, buturous, membranous, moist, slimy, soft, tough, viscid, waxy.AGAR COLONIES: age 16 da.

Macroscopic

Size: 2 mm.Shape: filamentous, irregular, oval, puntiform, round.Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.Topography: contoured, rough, smooth, striated, wrinkled.Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.Appearance by transmitted light: opaque, translucent, transparent.Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar Lt Ivory 2 ca

Potato slant

—
—

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.Surface growth: none, flocculent, membranous, pellicle, ring.Subsurface growth: none, granular, turbid.Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB: age _____

Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.

Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: Abundant growth.

Fat agar:

Glucose-nitrate agar: Growth. 12 da.Growth on nutrient agar better than on
— glucose— nutrient agar.Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.CATALASE: positive, negative.TEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +,
45°C. +, 55°C. -, 5°C. -, 50°C. +.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative.Sucrose: positive, negative.Xylose: positive, negative.Citrate: positive, negative.NH₄⁺ AS SOLE NITROGEN SOURCE: positive,
negative, slight.Mannitol: Positive. acid.

REDUCTIONS:

Nitrate: NO₃⁻ _____, NH₄⁺ _____, gas _____, negative 12 da.Methylene blue: positive, negative.Selenite: positive, negative.Tellurite: positive, negative. 4 da.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid _____, alkaline _____, neutral, gas.Sucrose: acid _____, alkaline _____, neutral, gas.Lactose: acid _____, alkaline _____, neutral, gas.Xylose: acid _____, alkaline _____, neutral, gas.Mannitol: acid _____, alkaline _____, neutral, gas.

HYDROLYSIS:

Gelatin: positive, negative. 3 da.Casein: positive, negative. 3 da.Fat: positive, negative.Starch: positive, negative 14 da.Cellulose: positive, negative.Urea: positive, negative 11 da.

TOLERANCES:

Salt: 2%—positive, negative. 1 da.7%—positive, negative. 1 da.10%—positive, negative. 6 da.pH: acid _____, alkaline _____.

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral. 1 da.Curd: acid, alkaline, absent, gas. 89 da.Peptonization: positive, negative. 22 da.Reduction: positive, negative. 7 da.

OTHER REACTIONS:

H₂S from _____: positive,
negative.NH₄⁺ from peptone: positive, negative.Acetylmethylcarbinol: positive, negative. 12 da.Indol: positive, negative 12 da.Methyl red: positive, negative.

Descriptive Chart

<u>277a-2</u> (code number)	<u>Trypticase Soy Agar</u> (medium)	<u>Chile Atacama Desert</u> (source)
<u>Bacillus coagulans</u> (name of organism)	<u>28°C.</u> (temperature)	<u>W.B. Bollen & K.M. Kemper</u> (studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round,
filaments, cocci, spirals,
branching _____

Size: average —
range —

Irregular forms:

GRAM REACTION: **Positive.**

18 hrs:

24 hrs:

48 hrs:

NIGROSIN STAIN:



1000X

PASTEURIZATION SURVIVAL (85°C, 10 minutes): **Positive.**

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

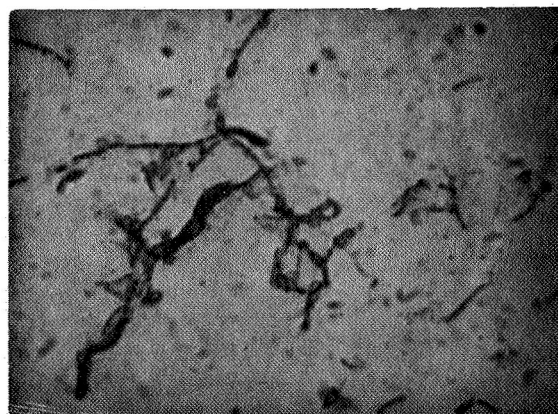
Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —
range —

MOTILITY: age _____.

Flagella:

SPORE STAIN:



1000X

OTHER STAINS:

Acid fast:

Capsule:

Glycogen:

Crystalline dextrans:

Fat globules:

Metachromatic granules:

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 14 da.Amount of growth: abundant, moderate, scant.Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.Consistency: adherent, brittle, butyrus, membranous, moist, slimy, soft, tough, viscid, waxy.AGAR COLONIES: age 16 da.

Macroscopic

Size: 3 mm.Shape: filamentous irregular, oval, puntiform, round.Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.Topography: contoured, rough, smooth, striated, wrinkled.Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate fimbriate.Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.Appearance by transmitted light: opaque, translucent, transparent.Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar Bamboo 2 gc

Potato slant

-

-

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.Surface growth: none, flocculent, membranous, pellicle, ring.Subsurface growth: none, granular, turbid.Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB: age _____

Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: Moderate growth.

Fat agar:

Glucose-nitrate agar: No Growth. 12 da.Slight growth on nutrient agar; no growth on glucose-nutrient agar.Anaerobic nitrate broth: no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, microaerophilic.CATALASE: positive, negative.TEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +, 45°C. +, 55°C. -, 50°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative.Sucrose: positive, negative.Xylose: positive, negative.Citrate: positive, negative.NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative.Mannitol: Negative.

REDUCTIONS:

Nitrate: NO₃⁻ _____, NH₄⁺ _____, gas _____, negative.Methylene blue: positive, negative.Selenite: positive, negative.Tellurite: positive, negative.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: Sacid, Q, alkaline, neutral, gas. ^cSucrose: acid, alkaline, neutral, gas.Lactose: acid, alkaline, neutral, gas.Xylose: acid, alkaline, neutral, gas.Mannitol: acid, alkaline, neutral, gas.

HYDROLYSIS:

Gelatin: positive, negative.Casein: positive, negative.Fat: positive, negative.Starch: positive, negative.Cellulose: positive, negative.Urea: positive, negative, growth

TOLERANCES:

Salt: 2% positive, negative.7% positive, negative.10% positive, negative.pH: acid, alkaline.

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral.Curd: acid, alkaline, absent, gas.Peptonization: positive, negative.Reduction: positive, negative.

OTHER REACTIONS:

H₂S from _____: positive, negative.NH₄⁺ from peptone: positive, negative.Acetylmethylcarbinol: positive, negative.Indol: positive, negative.Methyl red: positive, negative.

Descriptive Chart

<u>277a-1</u> (code number)	<u>Trypticase Soy Agar</u> (medium)	<u>Chile Atacama Desert</u> (source)
<u>Bacillus badius</u> (name of organism)	<u>28°C.</u> (temperature)	<u>W.B. Bollen & K.M. Kemper</u> (studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round,
filaments, cocci, spirals,
branching _____

Size: average —
range —

Irregular forms:

NIGROSIN STAIN:



1000X

GRAM REACTION **Positive.**

18 hrs:

24 hrs:

48 hrs:

PASTEURIZATION SURVIVAL (85°C, 10 minutes): **Positive.**

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

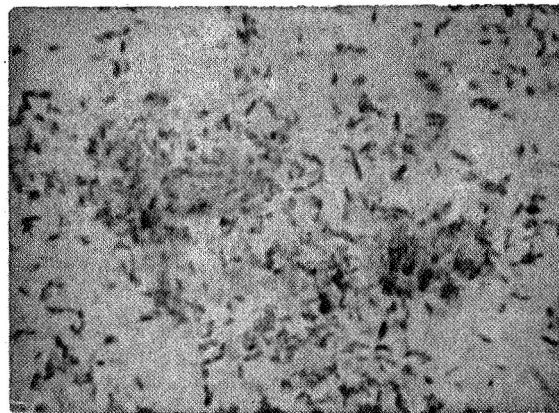
Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —
range —

SPORE STAIN:



1000X

MOTILITY: age _____

Flagella:

OTHER STAINS:

Acid fast:

Capsule: **Positive.**

Glycogen:

Crystalline dextrans:

Fat globules:

Metachromatic granules:

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 14 da.Amount of growth: *abundant, moderate, scant.*Form: *aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.*Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*AGAR COLONIES: age 16 da.

Macroscopic

Size: 3 mm.Shape: *filamentous, irregular, oval, puntiform, round.*Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*Topography: *contoured, rough, smooth, striated, wrinkled.*Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*Appearance by transmitted light: *opaque, translucent, transparent.*Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

(medium)

(color)

(CHM No.)

Trypticase soy agar

Camel**31e**

Potato slant

—

—

NUTRIENT BROTH: age _____

Amount of growth: *abundant, moderate, scant.*Surface growth: *none, flocculent, membranous, pellicle, ring.*Subsurface growth: *none, granular, turbid.*Sediment: *none, compact, flaky, flocculent, granular, viscid.*Odor: *resembling _____*

GELATIN STAB: age _____

Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: **No growth.**

Fat agar:

Glucose-nitrate agar: **No growth. 12 da.****Growth slight on nutrient agar; no growth on glucose-nutrient agar.****Anaerobic nitrate broth. Gas. 5 da.**

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: *aerobic, anaerobic, facultative, micro-aerophilic.*CATALASE: *positive, negative.*TEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 25°C. +, 30°C. +, 45°C. +, 55°C. -, 50°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: *positive, negative.*Sucrose: *positive, negative.*Xylose: *positive, negative.*Citrate: *positive, negative.*21 da.NH₄⁺ AS SOLE NITROGEN SOURCE: *positive, negative.*14 da.**Mannitol: Negative.**14 da.

REDUCTIONS:

Nitrate: NO₃⁻ +, NH₄⁺ _____, gas _____, *negative.*5 da.Methylene blue: *positive, negative.*16 da.Selenite: *positive, negative.*11 da.

—

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: *acid, alkaline, neutral, gas.*Sucrose: *acid, alkaline, neutral, gas.*Lactose: *acid, alkaline, neutral, gas.*Xylose: *acid, alkaline, neutral, gas.*Mannitol: *acid, alkaline, neutral, gas.*

HYDROLYSIS:

Gelatin: *positive, negative.*3 da.Casein: *positive, negative.*31 da.Fat: *positive, negative.*Starch: *positive, negative.*14 da.Cellulose: *positive, negative.*60 da.Urea: *positive, negative. (no growth)*11 da.

TOLERANCES:

Salt: 2%—*positive, negative.*6 da.7%—*positive, negative.*11 da.10%—*positive, negative.*11 da.pH: *acid, alkaline.*

LITMUS MILK REACTIONS:

Reaction: *acid, alkaline, neutral.*89 da.Curd: *acid, alkaline, absent, gas.*89 da.Peptonization: *positive, negative.*89 da.Reduction: *positive, negative.*89 da.

OTHER REACTIONS:

H₂S from _____: *positive, negative.*NH₄⁺ from peptone: *positive, negative.*Acetylmethylcarbinol: *positive, negative.*12 da.Indol: *positive, negative.*12 da.Methyl red: *positive, negative.*

Descriptive Chart

275a	(#6-20d)	Trypticase Soy Agar	Chile Atacama Desert
(code number)		(medium)	(source)
<u>Bacillus firmus*</u>	28°C.	W.B. Bollen & K.M. Kemper	
(name of organism)	(temperature)	(studied by)	

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round,
filaments, cocci, spirals,
branching _____

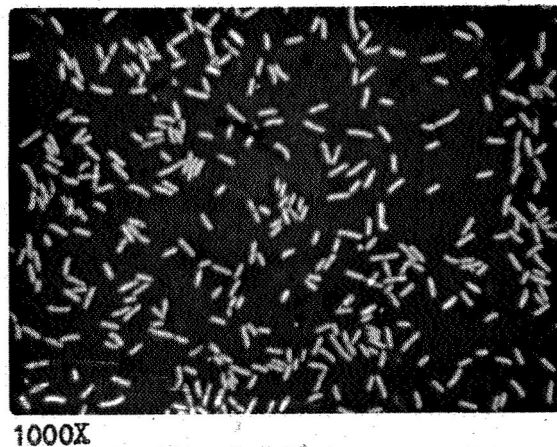
Size: average —
range —

Irregular forms:

NIGROSIN STAIN:

GRAM REACTION: Positive.

18 hrs:
24 hrs:
48 hrs:



PASTEURIZATION SURVIVAL (85°C, 10 minutes): Positive.

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.
Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —
range —

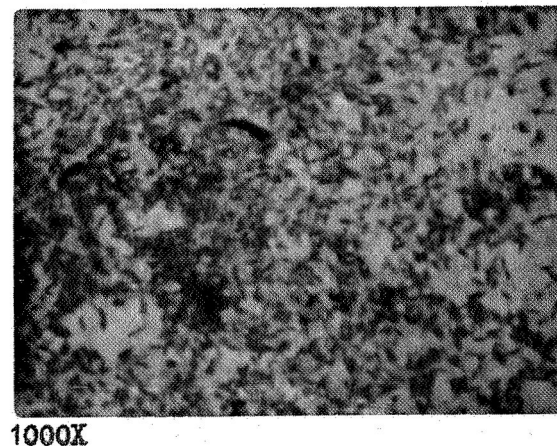
SPORE STAIN:

MOTILITY: age _____.

Flagella:

OTHER STAINS:

Acid fast:
Capsule:
Glycogen:
Crystalline dextrans:
Fat globules:
Metachromatic granules:



*Only difference is casein hydrolysis. This isolant has no casein hydrolysis after 30 days; B. firmus has moderate to wide zone of hydrolysis.

II. CULTURAL CHARACTERISTICS

(#6-20d)

AGAR STROKE: age 14 da.Amount of growth: abundant, moderate, scant.Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.AGAR COLONIES: age 14 da.

Macroscopic

Size: 2 mm.Shape: filamentous, irregular, oval, puntiform, round.Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.Topography: contoured, rough, smooth, striated, wrinkled.Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.Appearance by transmitted light: opaque, translucent, transparent.Medium: blackened, blueed, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium)

(color)

(CHM No.)

Trypticase soy agar Cinnamon3 le

Potato slant

—

—

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.Surface growth: none, flocculent, membranous, pellicle, ring.Subsurface growth: none, granular, turbid.Sediment: none, compact, flaky, flocculent, granular, viscid.Odor: resembling _____.

GELATIN STAB: age _____

Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: No growth.

Fat agar:

Glucose-nitrate agar: No growth. 12 da.Growth on nutrient agar; no growth on — glucose-nutrient agar.Anaerobic nitrate broth; no gas. 12 da.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.CATALASE: positive, negative.TEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 30°C. +, 40°C. +, 45°C. +, 55°C. -, 5°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative.Sucrose: positive, negative.Xylose: positive, negative.Citrate: positive, negative.21 da.NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative.14 da.

REDUCTIONS:

Nitrate: NO₃⁻ +, NH₄⁺ _____, gas _____, negative. 5 da.Methylene blue: positive, negative.Selenite: positive, negative. slight. 16 da.Tellurite: positive, negative. 11 da.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid, alkaline, neutral, gas, o&cSucrose: acid, alkaline, neutral, gas.Lactose: acid, alkaline, neutral, gas.Xylose: acid, alkaline, neutral, gas.Mannitol: acid, alkaline, neutral, gas.

HYDROLYSIS:

Gelatin: positive, negative.Casein: positive, negative. 31 da.Fat: positive, negative.Starch: positive, negative. 6 da.Cellulose: positive, negative. 60 da.Urea: positive, negative. (no growth) 11 da.

TOLERANCES:

Salt: 2%—positive, negative.7%—positive, negative.10%—positive, negative.pH: acid, alkaline

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral.Curd: acid, alkaline, absent, gas.Peptonization: positive, negative.Reduction: positive, negative.

OTHER REACTIONS:

H₂S from _____: positive, negative.NH₄⁺ from peptone: positive, negative.Acetylmethylcarbinol: positive, negative.Indol: positive, negative.Methyl red: positive, negative.

Descriptive Chart

275a-2 (#6)

(code number)

Trypticase Soy Agar

(medium)

Chile Atacama Desert

(source)

Bacillus firmus*

(name of organism)

28°C.

(temperature)

W.B. Bollen & K.M. Kemper

(studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends round,
filaments, cocci, spirals,
branching _____

Size: average —
range —

Irregular forms:

NIGROSIN STAIN:

GRAM REACTION: **Positive.**

18 hrs:

24 hrs:

48 hrs:



1000X

PASTEURIZATION SURVIVAL (85°C, 10 minutes): **Positive.**

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —
range —

MOTILITY: age _____.

Flagella:

OTHER STAINS:

Acid fast:

Capsule: **Positive.**

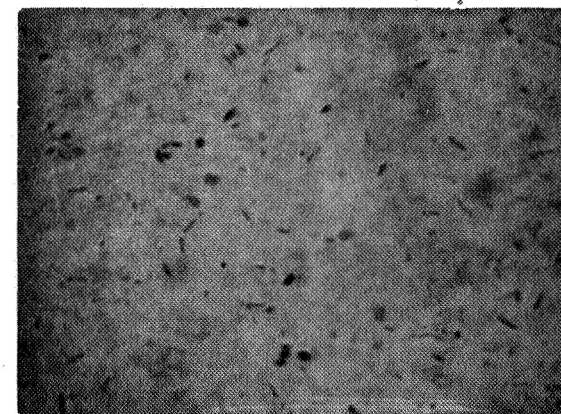
Glycogen:

Crystalline dextrans:

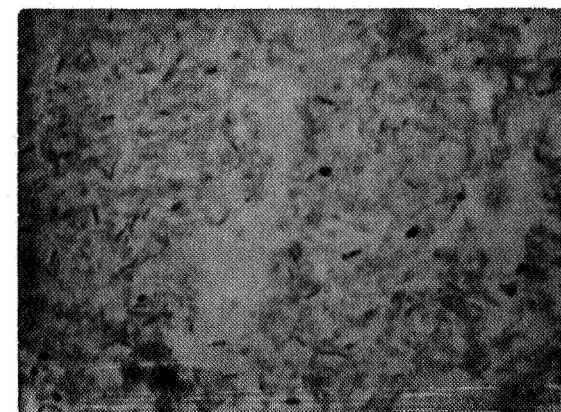
Fat globules:

Metachromatic granules:

SPORE STAINS:



1000X



1000X

*Only difference is casein hydrolysis. This isolant has no casein hydrolysis after 31 days.

II. CULTURAL CHARACTERISTICS

(46)

AGAR STROKE: age 13 da.Amount of growth: *abundant, moderate, scant.*Form: *aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.*Consistency: *adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.*AGAR COLONIES: age 13 da.

Macroscopic

Size: 2 mm.Shape: *filamentous, irregular, oval, puntiform, round.*Elevation: *beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.*Topography: *contoured, rough, smooth, striated, wrinkled.*Habit: *compact, spreading.*

Microscopic (100x)

Margin: *Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.*Internal structure: *amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.*

Optical properties

Appearance by reflected light: *dull, fluorescent, glistening, iridescent, opalescent.*Appearance by transmitted light: *opaque, translucent, transparent.*Medium: *blackened, blued, browned, grayed, greened, yellowed, unchanged.*

Chromogenesis:

(medium)

(color)

(CHM No.)

Trypticase soy agar Cinnamon3le

Potato slant

—

—

NUTRIENT BROTH: age _____

Amount of growth: *abundant, moderate, scant.*Surface growth: *none, flocculent, membranous, pellicle, ring.*Subsurface growth: *none, granular, turbid.*Sediment: *none, compact, flaky, flocculent, granular, viscid.*

Odor: resembling _____

GELATIN STAB: age 73 da.Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.*Rate: *fast, moderate, slow.*

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: **No growth.**Fat agar: **No growth.**Glucose-nitrate agar: **No growth. 12 da.****Slight growth on nutrient agar; no growth on glucose-nutrient agar.****Anaerobic nitrate broth: no gas. 12 da.**

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: *aerobic, anaerobic, facultative, micro-aerophilic.*CATALASE: *positive, negative.*TEMPERATURE RELATIONSHIPS: age 17 da.Growth at 10°C. +, 20°C. +, 35°C. +, 45°C. +, 55°C. -, 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: *positive, negative.*Sucrose: *positive, negative.*Xylose: *positive, negative.*Citrate: *positive, negative.*21 da.NH₄⁺ AS SOLE NITROGEN SOURCE: *positive, negative.*14 da.

REDUCTIONS:

Nitrate: NO₂⁻ +, NH₄⁺ _____, gas _____, *negative.* 5 da.Methylene blue: *positive, negative.*Selenite: *positive, negative.*Tellurite: *positive, negative.*11 da.

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: *acid, alkaline, neutral, gas.* o&cSucrose: *acid, alkaline, neutral, gas.*Lactose: *acid, alkaline, neutral, gas.*Xylose: *acid, alkaline, neutral, gas.*Mannitol: *acid, alkaline, neutral, gas.*

HYDROLYSIS:

Gelatin: *positive, negative.* slight.14 da.Casein: *positive, negative.*30 da.Fat: *positive, negative.*Starch: *positive, negative.*6 da.Cellulose: *positive, negative.*Urea: *positive, negative.* (no growth)11 da.

TOLERANCES:

Salt: 2%—*positive, negative.*1 da.7%—*positive, negative.*9 da.10%—*positive, negative.*11 da.pH: *acid, alkaline.*

LITMUS MILK REACTIONS:

Reaction: *acid, alkaline, neutral.*89 da.Curd: *acid, alkaline, absent, gas.*89 da.Peptonization: *positive, negative.*89 da.Reduction: *positive, negative.*89 da.

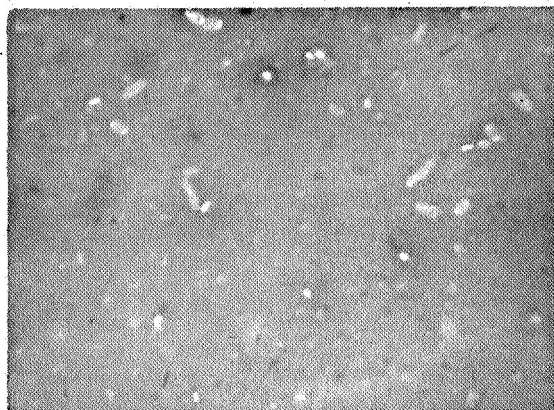
OTHER REACTIONS:

H₂S from _____: *positive, negative.*NH₄⁺ from peptone: *positive, negative.*Acetylmethylcarbinol: *positive, negative.*12 da.Indol: *positive, negative.*12 da.Methyl red: *positive, negative.*

PHOTOMICROGRAPHS OF NON-VIABLE ISOLANTS

Nigrosin stains of original cultures.

245Aa (#24)



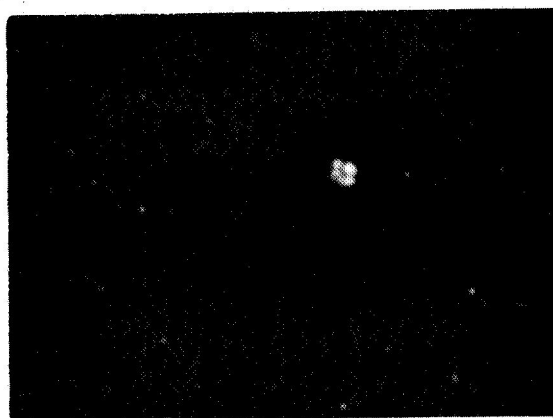
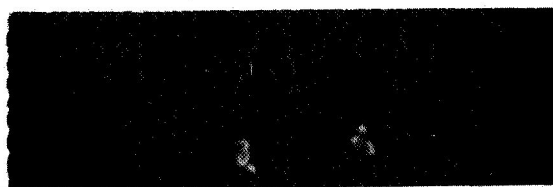
1000X

245Be (#24)



1000X

248Ab (#24)



1000X

246b (#25)



1000X

259a (#26)



1000X

260a (#26)



1000X

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